TEMPORAL AND SPATIAL DIFFERENCES IN SMOLTING AMONG SOCKEYE SALMON (*ONCORHYNCHUS NERKA*) POPULATIONS THROUGHOUT FRESH- AND SEAWATER MIGRATION AND THE EFFECT OF WATER TEMPERATURE ON THE SMOLT WINDOW

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ABSTRACT

Salmon smolts undergo physiological changes in the spring that are important for successful migration to seawater. Species that are widely distributed may differ in timing of physiological changes associated with smolting. In my first study, I compared indices of smolt characteristics among populations that differ in migration distance to the ocean. Fraser River sockeye salmon from four regions in the watershed were intercepted at different times during migration to characterize the parr-smolt transformation. Gill Na^+/K^+-ATPase (NKA) activity was highly variable at the start of migration, and was not explained by the distance from the ocean. Gill NKA activity changes with migration were also highly variable, but consistently smolts in the ocean had the highest gill NKA activities. The nature of smolting appears to be dynamic and variation was not based on the region of origin, timing during migration, or on the year of migration. The duration of time when anadromous salmon are able to survive in seawater – the smolt window – is influenced by temperature. In my second study, I found that warm water temperature abbreviated the smolt window. Additionally, isoforms of the gill NKA enzyme and endocrine signals suggest that the stimulus for smolting occurred prior emigration from the natal lake. Modeling the thermal experience that smolts encountered as they migrated downstream to the ocean in 2012 suggested Chilko fish did not experience temperatures as warm as the temperatures that abbreviated the smolt window in my study. Furthermore, climate change projections for temperature may not limit successful emigration of Chilko sockeye salmon smolts from central British Columbia to the ocean – but changes in other abiotic and biotic factors may confound this prediction.

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PROLOGUE

The life cycle of anadromous Pacific salmon involves movements between freshwater and seawater habitats. These fish have adapted to use fresh water in the early phases of the lifecycle, but also to maximize growth opportunities in the productive ocean as adults. Sockeye salmon (*Oncorhynchus nerka*) generally make more use of lake-rearing habitat in juvenile stages than other species of Pacific salmon (Burgner, 1998). Sockeye salmon eggs are laid in gravel riverbeds. The following spring, after absorption of the yolk sac, sockeye salmon fry will emerge from the spawning gravel and migrate to nearby natal lakes. The majority of juvenile sockeye salmon spend 1-2 years (Burgner, 1998; Hoar, 1988) in the freshwater lake before making the downstream migration to the ocean. There they spend the next 1-2 years feeding in the more productive marine habitat until environmental cues signal the next phase, return to freshwater and upriver migration to spawning locations. During freshwater migration, fish cease feeding, mature sexually, and transform from slender, silver salmon to brilliant red spawners. Males develop sexual morphological traits such as a prominent dorsal hump and elongated jaws. Sockeye salmon return to their natal grounds with remarkable precision both in terms of spawning location and migration timing (Quinn et al., 1999; Dittman and Quinn, 1996). The ability to survive in both freshwater and seawater make salmon fascinating, however, the transition between environments is complex and requires a multitude of processes for successful ocean entry (McCormick, 2013) and return to fresh water (Shrimpton, 2013).

The preparatory phase for successful acclimation to a saltwater environment is known as smolting, where freshwater parr transform into seawater tolerant smolts. The more obvious changes in the parr-smolt transformation are morphological and behavioural. Morphological changes occur when external parr marks become less distinct

(through the thickening purine layer; Hoar, 1988), the body changes colour, becoming more silver with a darker back, and it also becomes more slim and stream-lined (Burgner, 1998). Behaviourally, fish become less territorial and increase schooling. As well, changes in swimming orientation occur, where fish orient themselves downstream to actively migrate to the ocean, likely a predatory avoidance adaptation (Hinch et al., 2006). Although, transitioning to survive in the ocean involves a complex suite of adaptations, physiological changes that enable smolts to ionoregulate in seawater are arguably the most important (McCormick, 2013). Anadromous salmon have the ability to tolerate seawater and fresh water by regulating a constant internal osmotic concentration, ~30% of the NaCl concentration of seawater (Evans et al., 2005).

The length of time that juvenile salmon are able to survive this transfer to seawater is referred to as the smolt window. If smolts are prevented from reaching seawater within this smolt window, there is a loss of the characteristics associated with marine survival and smolts revert back to a freshwater state (Hoar, 1988; McCormick et al., 1997; 1999). Saltwater exposure after the smolt window can be fatal for a fish, as they lack the physiological machinery to regulate ions in a hyperosmotic environment.

The parr-smolt transformation is stimulated by environmental factors that change during the spring – predominantly photoperiod (Clarke et al., 1978) and temperature (McCormick et al., 2000). Temperature, however, does not stimulate smolting in the absence of a photoperiod signal (McCormick et al., 2002). Other factors have also been shown to influence smolting. Flow has been linked to the timing of migration (Sykes et al., 2009) and a directional current leads to a more defined downstream swimming behaviour (Sykes and Shrimpton, 2010). There is also evidence that turbidity will alter

physiological development associated with hypo-ionoregulatory ability (Shrimpton et al., 2007). Changing environmental conditions during the spring, however, may affect migration and compromise successful transition to seawater. In seawater, feeding habits alter and predation increases (McCormick et al., 1998). Fish not only need to survive the transition to seawater, but need to be healthy enough to cope with the new environment. Therefore, fish that are accelerated or delayed beyond the boundaries of the smolt window are at risk. The margins of the smolt window may capture the survival of transference between habitats, but deviations from the peak optimal hyper osmoregulation may be detrimental.

Anthropogenic changes to the landscape affect the environmental cues that regulate smolting. Whereas, photoperiod is predictable and stable, annual fluctuations in water temperature, flow and turbidity exist. Changes to the environment can influence these factors; the shifting temperatures in our climate are altering these cues (Hague et al., 2011; Jonsson and Jonsson, 2009; Reed et al., 2011). Warming temperatures have been observed as a result of climate change and may expose smolts to warmer temperatures earlier in the season. The maximum Fraser River water temperature increased by approximately 2 °C in the last 60 years (Patterson et al., 2007), and summer mean water temperature for the period 2070-2099 is predicted to increase a further 1.9 °C (Morrison et al., 2002). Along with warming temperatures, timing of spring freshet is predicted to change (Morrison et al., 2002), and smolts may experience higher velocity and more volume of water sooner than when fish are ready to reach seawater. Similarly, direct alterations to the landscape will alter the subtleties of the environment. For example, dams can affect the upstream and downstream temperatures by halting flow and allowing

for thermal stratification of the water column (Cushman, 1985; Binsted and Ashley, 2006). As well, dams alter flow to fit supply and demand, as opposed to natural fluctuations in run-off (McCartney et al., 1999). Dramatic physical changes occur, where rivers are altered to lake-like reservoirs. Turbidity is altered as an effect of flow, but also production of reservoirs increase erosion, introducing more suspended sediments into a water system. However, impoundments that alter the flow of a system eventually cause suspended sediments to settle out. In the case of smolting, environmental cues will be changing with anthropogenic developments, and this fragile period in the salmon's lifecycle needs to be considered while making management decisions.

It has been suggested that climate change will cause warming temperatures and more variable flows for river systems including the Fraser River (Hague et al., 2011; Morrison et al., 2002; Reed et al., 2011). If lower flows align with outmigration timing, then juvenile migration time will be longer and in warmer water – potentially putting populations of salmon at risk. Atlantic salmon held at warmer temperatures showed an abbreviated smolt window and a rapid loss of smolt characteristics with warmer temperatures (McCormick et al., 1999). In addition, the decrease in flow may delay migration and lengthen the time required for smolts to reach the ocean – an environment for which they have already begun to adapt. If the smolt window is affected by temperature, then fish could potentially lose their adaptations to a hyperosmotic environment prior to seawater entry, causing high mortality or loss of smolt characteristics.

Sockeye salmon are a tremendous natural resource for the province of British Columbia. The Fraser River is the world's largest producer of wild sockeye salmon

(Cooke et al., 2004). This fishery contributed approximately CAD$172.3 million of wholesale value to the BC economy as a result of record high returns of 34 million sockeye salmon to the Fraser system in 2010 (Province of BC, 2010). However, the Fraser sockeye salmon stocks are not consistent in annual returns. For example, in 2009 sockeye salmon returns were at a record 60 year low of 1.3 million (Pacific Salmon Commission, 2009) which lead to the Cohen Commission, a federal inquiry into the status of Fraser River sockeye salmon. The Fraser sockeye salmon fishery is comprised of three distinct harvest sectors: the Aboriginal communal fishery, the general commercial fishery, and the recreational fishery (Cohen, 2012).

The importance of sockeye salmon to the province of BC far exceeds their monetary value. Culturally, salmon play an important role in the spirituality of Aboriginals on the Pacific Coast. Aboriginal communal fishing licenses authorize fishing for food, social, and ceremonial purposes. In respect of fishing for these purposes, Aboriginal groups have a priority of access to Fraser River sockeye salmon, second only to conservation (Cohen, 2012). The recreation fishery also provides exciting opportunities, food and social activity to anglers. The commercial fishery provides jobs and contributes strongly to the economy of the province. As well, ecologically, salmon contribute nutrients essential to natal lakes and spawning tributaries (Naiman et al., 2002; Johnston, 2004). A healthy salmon return is, therefore, important to sustain the population of this species as well as the other species that are dependent on the sustenance spawning carcasses provide (Cederholm et al., 2004). The value of sockeye salmon to the province provides further rationale for research to understand the complexity of their lifecycle.

The Fraser River watershed covers the majority of the southern interior of BC, and drains 220,000 km^2. Approximately 90 sockeye salmon spawning populations have been identified in the Fraser River drainage, and it is estimated that juveniles rear in 24 natal lakes. However, approximately 90 percent of the production is centered on fewer than 10 natal lakes (Beacham et al., 2004). The populations of returning adults have characteristic marine exit times and these are used broadly to classify Fraser River sockeye salmon populations into four groups for fishery management purposes. The Early Stuart run has the earliest returning spawners. The Early Summer run is composed of populations with widespread distributions, although are not abundant. The Summer run grouping exhibits abundant returns and the major populations are in the mid Fraser. The late run timing group is also abundant and populations can be fairly widespread in the Fraser River drainage. These grouping are associated with timing of adult migration, however a geographic distinction may be more appropriate for the juveniles. These four geographic regions are; the Upper Fraser, the Lower Mid-Fraser, the Lower Fraser and the Thompson (Beacham et al., 2004). These regional groupings, therefore, are a useful designation for making comparisons among juveniles from different sockeye salmon populations.

Smolt migration occurs over a fairly predictable interval of time in the spring throughout the geographically diverse Fraser River watershed. Whether differences in development of saltwater tolerance exist among populations that differ in distance to the ocean, temporally during the spring, or as fish migrate downstream for a species with wide geographic distribution is not known. It is important to understand variation that may occur, to have a more complete picture of the physiological processes that are

occurring for Fraser River sockeye salmon during downstream migration. In the first chapter, I examined the variation through spatial and temporal analysis of smolt characteristics of migrating juvenile sockeye salmon smolts in the Fraser watershed.

The Chilko Lake population is an indicator stock used to estimate smolt to adult survival for Fraser River sockeye salmon. As a result Fisheries and Ocean Canada (FOC) can partition out egg to smolt, and smolt to adult survival. As well, tagging studies on this stock are currently underway to further understand the survival during their downstream and early marine survival (Rechisky et al., 2013; Clark et al., submitted). In chapter 2, using this indicator stock, I performed a temperature manipulation experiment on migrating juveniles. Temperature has shown to have an effect on the smolt window (reviewed in McCormick, 2013), however, the effect has yet to be studied in Fraser River sockeye salmon. The Chilko population is a strong contributor to the Fraser run. Therefore, environmental stresses such as temperature may have strong implications for the Fraser run of sockeye salmon.

This thesis aims to shed light on the physiological processes that occur during the parr-smolt transformation. The diversity in location and timing of smolt migration and potential relationships in smolt physiology was examined. As well, the potential effects of warming temperature on the smolt window were also examined. Information on this crucial time of a sockeye salmon lifecycle will give more insight on potential effects as a result of changes to the thermal environment.

CHAPTER 1

Temporal and spatial differences in smolting among sockeye salmon (*Oncorhynchus nerka*) populations throughout fresh- and seawater migration

ABSTRACT

Physiological changes that occur in the spring are preparatory for salmon smolts to successfully enter seawater, but variation is likely to exist within species with a wide geographic distribution. Whether differences in development of saltwater tolerance exist among populations that differ in distance to the ocean, temporally during the spring, or as fish migrate downstream is not known. Juvenile sockeye salmon from four regions in the Fraser River Watershed, British Columbia, were intercepted to assess physiological differences among populations and at different times during migration to characterize the parr-smolt transformation. Pre-migratory fish had low levels of gill Na^+/K^+-ATPase (NKA) activity. However, high gill NKA activities were observed at the start of migration for some populations, smolts leaving the lake did not consistently have higher gill NKA activity than non-migratory juvenile sockeye salmon sampled in their natal lakes. Gill NKA was highly variable at the start of migration with no relationship to distance from the ocean. Gill NKA activity changes with migration were also highly variable, but consistently smolts in the ocean had the highest gill NKA activities. Internal and external factors may influence this variation, but the dynamic nature of smolting was not based on the region of origin, timing during migration, or on the year of migration.

INTRODUCTION

Sockeye salmon (*Oncorhynchus nerka*) make more use of lake-rearing habitat than any of the other *Oncorhynchus* species during juvenile stages (Burgner, 1998). Following absorption of the yolk sac, the majority of sockeye salmon fry emerge from the spawning gravel and migrate to

a resident nursery lake where juveniles spend 1-2 years (Burgner, 1998; Hoar, 1988) before making the downstream migration to the ocean. A preparatory transformation known as smolting occurs before seawater entry that increases survival within the marine environment. A complex suite of morphological, behavioural, biochemical and physiological changes occur during the transformation from parr to smolt that has been well described for anadromous salmon (McCormick and Saunders, 1987). Important aspects of smolting are the ability of the fish to ionoregulate in both seawater and fresh water and maintain a constant internal osmotic concentration (Evans et al., 2005). It is important for animals to maintain their internal ionic concentration within a relatively narrow range, as deviations from this range lead to poor performance or mortality (Brauner et al., 1992; Shrimpton et al., 1994). Remarkably, physiological changes associated with the parr-smolt transformation enable juveniles to migrate from fresh water to the marine environment with minimal perturbation (McCormick and Saunders, 1987).

Physiological changes that occur in salmon smolts in fresh water are adaptive for seawater entry (McCormick and Saunders, 1987; McCormick et al., 1999), but are reversible if smolts do not enter seawater (reviewed in McCormick et al., 1997). The transient nature of smolting, therefore, requires that development of saltwater tolerance be coincident with downstream migration and ocean entry – at least for coastal populations of anadromous salmon. Interior populations of salmon, however, must migrate far greater distances than coastal populations to reach the ocean and yet little is known regarding smolt physiology for interior salmon populations – particularly sockeye salmon which migrate directly from their natal lakes to the ocean. Further an understanding of smolting during migration is imperative as

anthropogenic alterations (such as temperature and water diversions) to the watershed will change the migration environment, and thus affect physiology of migrating fish.

The gill is important for ionoregulaton in both seawater and fresh water. It has specialized cells in the gill known as ionocytes that secrete excess ions in seawater and uptake ions in fresh water (Evans et al., 2005). In freshwater, fish must minimize loss of body ions to the hypo-osmotic environment, whereas in seawater fish must replace water and excrete excess ions from the body. Within the ionocytes, enzymes and transporter proteins are important for maintaining ion balance. The primary protein involved in both freshwater and saltwater ionoregulation is the enzyme Na^+/K^+-ATPase (NKA). Specifically it is different isoforms of the enzyme that are important for ionoregulation in the two media (Richards et al., 2003). NKA activity also increases in higher salinity water (McCormick, 1995; McCormick et al., 1998) and the activity of this enzyme in the gill is a strong indicator of ionoregulatory capacity in seawater. Gill NKA activity, therefore, is used as a marker of seawater tolerance and smolting in anadromous salmon.

The length of time that juvenile salmon are able to survive transfer to seawater is known as the smolt window. If smolts are prevented from reaching seawater within the smolt window, there is a loss of saltwater tolerance and smolts revert back to a freshwater form (Hoar, 1988; McCormick et al., 1997; 1999). Saltwater exposure after the smolt window can be fatal for a fish, as they lack the physiological machinery to regulate ions in a hyperosmotic environment. The duration of the smolt window has been linked to temperature (McCormick et al., 1997), but whether there are species-specific differences or even population level differences within a species is not known. Characterizing differences in the smolt window among different

populations from a large watershed will provide a better understanding of factors controlling smolting – particularly in light of potential impacts of climate change on physiological function.

Research questions

The Fraser River watershed is the largest catchment in British Columbia and has a tremendous diversity of salmon habitats. For example, within the Fraser system, sockeye salmon rear in coastal lakes that drain into the lower river (Cultus Lake), interior lakes from southern BC (Shuswap Lake), to lakes in north central BC (Chilko Lake). Such a vast watershed offers an opportunity to compare among populations and assess how smolt physiology may be affected spatially and temporally among populations to answer three research questions.

Is there a stock difference in NKA activity in smolts at the start of migration? Smolts at the onset of migration appear seawater tolerant, however, this work has examined species and populations that have short distances to migrate before seawater entry. Whether migration distance to the ocean affects the development of seawater tolerance is not known. To examine the effect of migration distance on the development of smolt physiology, four populations of sockeye salmon were sampled at the start of migration as they left their natal lakes with varying downstream migration distances (113 km to 684 km).

Is there a temporal difference in NKA activity at any specific stage of migration? To examine temporal variation in smolt physiology, sockeye salmon were intercepted before migration from their natal lake, at the start of migration, and during migration.

Are there changes in NKA activity from the start of migration through to seawater entry? To examine this research question, populations from the Fraser Watershed were divided into four regional groups. Fish migrating at the peak of migration were caught to assess physiology in

their natal lakes before migration, the start of migration, prior to seawater entry, and migrants in the ocean were compared.

METHODS AND MATERIALS

Animals

Juvenile sockeye salmon were sampled in lakes, tributaries, and the main-stem of the Fraser River watershed, and also the coastal waters of British Columbia, Canada, in 2010, 2011 and 2012. Sampling was timed and locations were chosen to assess physiological differences among populations and at different times during the parr-smolt transformation.

Smolt physiology at the start of migration

Sockeye salmon smolts were intercepted as they began migration from lakes in the Fraser River watershed; Chilko Lake, Shuswap Lake, Anderson-Seton Lakes, and Cultus Lake (Fig. 1.1). Migration distance and elevation for each lake is; Chilko, 684 km and 1172 m, Shuswap Lake, 498 km and 360 m, Anderson-Seton Lakes 335 km and 243 m, Cultus is 113 km and 47 m. Chilko Lake smolts were collected in the Chilko River at the Fisheries and Oceans Canada (FOC) enumeration fence approximately 1 km downstream of Chilko Lake. A smolt sampling program below Shuswap Lake intercepted migrating smolts on the Little River. Smolts were collected approximately 1.9 km downstream of the where Adams River flows into Shuswap Lake, the majority of the population would be from the Late Shuswap population (David Patterson, personal communication). Smolts from Anderson and Seton Lakes were collected in the Seton River approximately 1 km downstream of Seton Dam with an inclined plane trap. The majority of fish intercepted in the Seton River would have been of Gates Creek origin (David Patterson, personal communication). Cultus Lake smolts were caught at the FOC enumeration fence on

Sweltzer Creek, approximately 300 m downstream of Cultus Lake. All fish were sampled near the peak of migration (Table 1.1).

Temporal variation in smolt physiology

Migrating sockeye salmon were intercepted on multiple dates at the same location to assess whether physiological status changed with timing of migration during the smolt run for two stocks. Chilko Lake smolts were sampled at the enumeration fence. Fish from the Shuswap stock were captured prior to migration in Shuswap Lake and also at the start of migration in the Little River. Migrating sockeye salmon smolts of Chilko and Shuswap origin were also intercepted in the Lower Fraser at Mission. These fish were identified by genetic analysis (see below); dates and number of fish captured are summarized in Table 1.2.

Physiological changes during migration

To characterize developmental changes that occurred during migration and whether there was a difference among stocks with distance from the ocean, sockeye salmon juveniles were sampled in their natal lake, during downstream migration to the ocean, and also in the ocean. For this research question, fish were separated into groups based on location within the Fraser watershed; Upper Fraser, Lower Mid-Fraser, Lower Fraser and the Thompson according to Beacham et al. (2004). The Upper Fraser group includes Stuart-Stellako, Bowron, Quesnel and Chilko populations. The Thompson group includes South and North Thomson River. The Lower Mid-Fraser includes the Portage and Gates Creek populations. The Lower Fraser group includes the regional groups; Lower Fraser (north side) and Lower Fraser (south side). A single lake system was targeted to collect smolts at the start of migration for each regional group (Table 1.3). For fish samples collected in the lower Fraser River and in the ocean, genetic analysis was used to identify population of origin for each fish (Beacham et al., 2004).

The methods used to collect fish were dependent on each sampling location. Pre-migratory fish were sampled in three locations. In the Chilko River, fry were collected as they migrated upstream along the margin of the river. In Shuswap Lake, pre-smolts were captured off Cruickshank point using a trawl. Juvenile sockeye salmon in Cultus Lake were also captured by trawl. Smolt capture methods at the start of migration were as described above.

In the lower Fraser River, migrating smolts were also captured just before saltwater entry. In 2010 and 2012, fish collected near Mission (79 km above tidal influence) were captured either in a rotary screw trap, incline plane trap, or a vertical trap. Traps were attached to a FOC vessel, and operated for 15 minutes at a time rotating from the north bank, to the center to the south bank of Fraser River. In 2011, fish were collected under the FOC Lower Fraser trawl program near the Port Mann Bridge (36 km upstream of Fraser Mouth) a location influenced by ocean tides, but with low salinity and still considered fresh water. Trawls occurred on the tide, and were approximately 10-minute trawl sets. For the Mission samples collected in 2010, 40% of the Upper Fraser fish came from the Stuart-Stellako system and 60% from the Chilko system, only seven fish were analyzed from the Lower Fraser and all were from Dolly Varden Creek (part of the Chilliwack Lake system). In 2011, only nine fish from the Upper Fraser group were analyzed, which was predominantly fish from the Stuart-Stellako system. Ten fish from the Thompson and no fish from either the Lower Mid-Fraser or the Lower Fraser groups were captured. In 2012, smolts analyzed for the Upper Fraser were Chilko, from the Thompson were Shuswap, and from the Lower Mid-Fraser were Gates stock (Table 1.3).

Ocean samples were collected in 2010, 2011 and 2012 with the FOC Strait of Georgia survey. In 2010, 60 fish were collected between 2-Jun to 7-Jun-2010. Of the 60 fish, 44 were designated as Upper Fraser; approximately 60% were from the Stuart-Stellako region, and 40%

from the Chilko system. Five fish from the Thompson watershed were collected. There was only one fish assigned to the Lower Mid-Fraser group, and it was of Gates Creek origin. Of the Lower Fraser group, seven of the 10 samples were from Dolly Varden Creek, whereas the other fish were from other Lower Fraser stocks; Birkenhead and Pitt (Table 1.3). In 2011, ocean samples were collected between 25-Jun to 2-Jul-2011. Most of the fish were from the Upper Fraser (38 fish), however nine fish from the Thompson and one fish from the both the Lower Mid-Fraser and Lower Fraser were analyzed (Nahatlatch Lake and Cultus Lake, respectively). In 2012, the majority of fish sampled were from Thompson stocks, also eight fish from the Upper Fraser, and one from the Lower Mid-Fraser were also analyzed.

Tissue Sampling

After capture, fish caught in fresh water were rapidly transferred to a bucket containing 200 mg · L^{-1} tricaine methanesulfonate buffered with 400 mg · L^{-1} sodium bicarbonate. Once fish were anesthetized, fork length (L) to the nearest mm and weight (W) to the nearest 0.01 g were measured. A portion of weight measurements were not taken due to inaccuracies by the scale because of poor weather conditions; May 10th, 2012 Shuswap Lake trawls and 2011 and 2012 Lower River samples do not include weight observations. Trawling in the ocean often brought fish on board post mortem, if gill tissue looked degraded, fish were omitted from tissue sampling. Fish still alive were euthanized by concussion. Fork length and weight were measured. For all fish, gill arches were removed and blotted to remove excess blood. Gill tissue from fish sampled in fresh water were immediately frozen on dry ice or liquid nitrogen for up to 30 days before transferring to -80 °C. Gill tissue from fish sampled in the ocean were submerged in 100 µL of SEI (150 mM sucrose, 10 mM Na_2EDTA, 50 mM imidazole, pH 7.3) and frozen in liquid nitrogen for up to 14 days before transferring to -80 °C.

Gill Na^+/K^+-ATPase activity

Gill NKA activity was measured according to the microassay protocol of McCormick (1993). In summary, gill filaments were homogenized in a SEI buffer (150 mM sucrose, 10 mM Na_2EDTA, 50 mM imidazole, pH 7.3) containing 0.1 % sodium deoxycholate (SEID). Centrifugation (5000 g for 30 seconds) produced the supernatant which was used to determine the enzyme activity by relating ATP hydrolysis to the oxidation of nicotinamide adeninedinucleotide (NADH), measured at 340 nm for 10 minutes at 25 °C, replicated twice in the presence and absence of 0.5 mmol · L^{-1} ouabain on a plate reader (VersaMax, Molecular Devices, Sunnyvale, California, USA). Protein content was then measured using a bicinchoninic acid (BCA) protein assay (Pierce, Rockford, IL). Specific activities were expressed as µmol ADP · mg^{-1} of protein · h^{-1}.

Statistical Analysis

Smolt physiology at the start of migration

Difference in NKA activity, length and condition factor (K; 100 x W· L^{-1}) were determined with analysis of variance (ANOVA). For the analysis of variables at the start of migration, samples were not obtained in all years for each study site; consequently comparisons were made among sites within a single year and within sites over multiple years in separate analyses. When significant differences were found, multiple comparisons of means were performed using a Tukey's test. In 2010 and 2011, both 1 and 2 year old smolts were collected at the Chilko River enumeration fence. Enzyme activities did not differ between the two age classes ($t=1.75$, $p=0.09$) and data was pooled. Older smolts were larger, but less numerous. Due to the small number of 2 year old smolts captured, size data was not included in the analysis.

Temporal variation in smolt physiology

To assess temporal variation in smolt physiology, an ANOVA was performed on NKA activity, length and K (when available) by the sample collection day for each capture location separately. When significant differences were found, multiple comparisons of means were performed using a Tukey's test.

Physiological changes with migration distance

Difference in NKA activity, length and K were determined with an ANOVA on group means among the different capture locations within each regional group. Additionally, where fish were sampled in multiple years at the same location, ANOVA was used to test for significant differences by location within a regional group. In the ocean samples, however, few fish were obtained for some of the regional groups and the mean across the three years of study was used for analysis. When significant differences were found, multiple comparisons of means were performed using a Tukey's test. Statistical significance was taken at a level of $p < 0.05$. All statistical analysis was performed in R software (ver. 3.0.2). All values are expressed as means ± 1 standard error (SE).

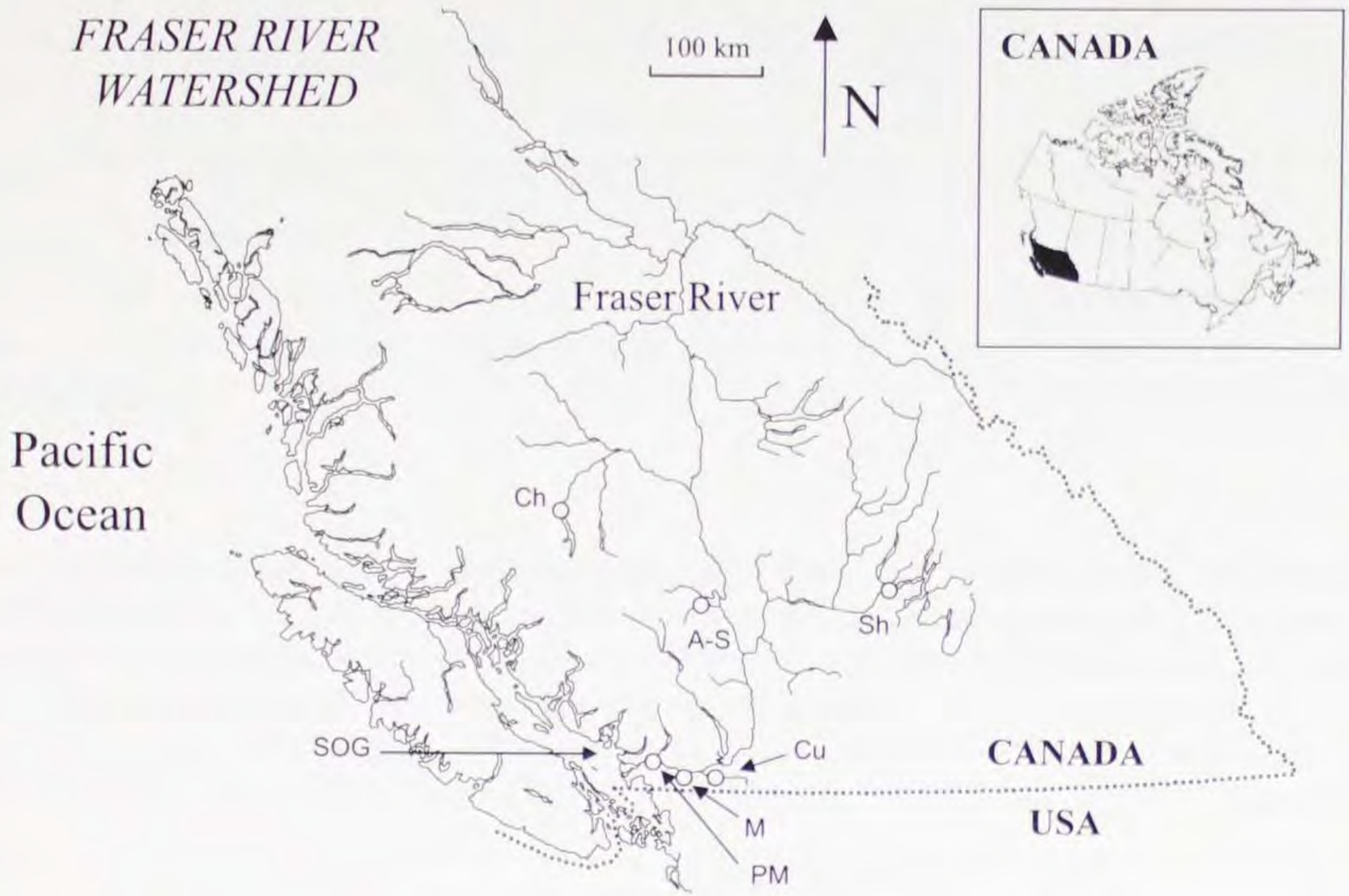

Figure 1.1: Map of the Fraser River watershed and coastal British Columbia, Canada showing sample locations for juvenile sockeye salmon. Four regional groups of sockeye were sampled in freshwater; Upper Fraser were fish from the Chilko River (Ch) watershed, Thompson were fish from Shuswap Lake (Sh), Lower Mid-Fraser were fish from the Anderson-Seton River (A-S) watershed, and Lower Fraser were fish from Cultus Lake (Cu). Pre-estuary sampling occurred at Mission (M) and at the Port Mann Bridge (PM). Seawater sampling occurred in the Strait of Georgia (SOG).

Table 1.1: Date migrating sockeye salmon were intercepted for four stocks from the Fraser River watershed. Fish were sampled near the peak of downstream migration. Sample size for each date is given in parentheses.

Stock	Capture Location	Year		
		2010	2011	2012
Chilko	Chilko River	6-May-2010 (11)	10-May-2011 (38)*	30-Apr-2012 (13)
Shuswap	Little River			24-May-2012 (12)
Anderson-Seton	Seton River			1-May-2012 (29)
Cultus	Sweltzer Creek	19-Apr-2010 (11)		3-Apr-2012 (24)

* n=28 for size data

Table 1.2: Date sockeye salmon were intercepted for Chilko and Shuswap stocks in the Fraser River watershed in 2012. Fish were sampled over multiple dates during the spring to assess temporal variation in smolt physiology. For Chilko stock, smolts were intercepted in the Chilko River below the lake and in the lower Fraser River at Mission. For Shuswap stock, sockeye salmon were sampled in the lake before migration, in the Little River downstream of Shuswap Lake, and in the lower Fraser River at Mission. Sample size for each date is given in parentheses.

Stock	Capture Location		
	Lake	Migration Start	Lower River
Chilko		21-Apr-2012 (11)	28-Apr-2012 (8)
		30-Apr-2012 (13)	2-May-2012 (7)
		8-May-2012 (11)	10-May-2012 (2)
		16-May-2012 (11)	22-May-2012 (1)
Shuswap	23-Apr-2012 (27)	3-May-2012 (12)	10-May-2012 (2)
	10-May-2012 (20)	10-May-2012 (11)	14-May-2012 (3)
		17-May-2012 (12)	22-May-2012 (3)
		24-May-2012 (12)	26-May-2012 (1)
		30-May-2012 (12)	30-May-2012 (12)
		6-Jun-2012 (12)	7-Jun-2012 (1)
		14-Jun-2012 (12)	

Table 1.3: Date sockeye salmon were intercepted at different locations during their migration in the Fraser River watershed and after ocean entry. Population of origin was determined by sampling location in lakes and upper rivers and by genetic analysis in the lower Fraser River and ocean. *indicates fish were sampled the previous year as listed here, but would be part of the same brood as the migrating smolts.

Region	Capture location			
	Pre-migration	Migration start	Lower river	Ocean
Upper Fraser				
	Chilko Lake	*Chilko River*	*Fraser River*	*Strait of Georgia*
2010		6-May (11)	6-May – 17-May (15)	2-Jun – 7-Jun (44)
2011		10-May (38)	24-May – 31-May (9)	25-Jun – 2-Jul (38)
2012	10-May* (9)	30-Apr (13)	28-Apr – 22-May (18)	12-Jul– 15-Jul (8)
Thompson				
	Shuswap Lake	*Little River*	*Fraser River*	*Strait of Georgia*
2010				4-Jun – 6-Jun (5)
2011			24-May (10)	25-Jun – 2-Jul (9)
2012	23-Apr (27)	24-May-2012 (11)	10-May – 7-Jun (22)	22-Jun – 15-Jul (103)
Lower Mid-Fraser				
	Anderson Lake	*Seton River*	*Fraser River*	*Strait of Georgia*
2010				4-Jun (1)
2011				29-Jun (1)
2012		1-May (29)	6-May – 22-May (27)	Jul-14 (1)
Lower Fraser				
	Cultus Lake	*Sweltzer Creek*	*Fraser River*	*Strait of Georgia*
2010	18-Mar (12)	19-Apr (11)	6-May – 17-May (7)	2-Jun – 7-Jun (10)
2011	2-Nov* (12)			25-Jun (1)
2012		3-Apr (24)		

RESULTS

Smolt physiology at the start of migration

There was considerable variation in gill NKA activity at the start of migration when juvenile sockeye salmon were leaving their natal lakes. My analysis indicated that NKA differed significantly among the four stocks sampled in 2012 (F_3=32.1, p=2.42x10^{-13}) (Fig. 1.2). NKA activity was significantly greater in Chilko smolts than the other stocks; Shuswap was intermediate and also differed from the other stocks; Anderson-Seton and Cultus were the lowest and did not differ. In contrast, NKA activities measured from Chilko and Cultus in 2010 did not differ significantly between the two stocks (t=0.37, p=0.72). For stocks sampled in multiple years, however, significant differences in gill NKA activity existed. For Chilko differences in NKA activities were observed across the three years of the study (F_2=7.23, p=1.48x10^{-3}). In 2010, NKA activity levels were significantly higher than the following two years; 2011 and 2012 samples did not differ significantly. At the start of migration for the Cultus stock, NKA activities were also significantly higher in 2010 than in 2012 (t=-9.28, p=1.03x10^{-10}).

There was also variation in length (L) of smolts at the start of migration. Length of smolts differed significantly among the four stocks sampled in 2012 (F_3=45.16, p=2x10^{-16}) (Table 1.4). Cultus smolts were significantly longer than the rest of the stocks. Chilko and Anderson-Seton did not differ significantly, but were longer than the Shuswap smolts. In 2010, length of smolts captured at Chilko and Cultus Lakes did not differ (t=1.78, p=0.09). There was also variability in length across years within stocks. For Chilko smolts, the 2012 smolts were considerably smaller (F_2=9.04, p=4.77x10^{-4}). The Cultus smolts were also significantly different between 2010 and 2012 (t=-3.84, p=5.25x10^{-4}).

Condition factor (K) differed significantly among the four stocks sampled in 2012 (F_3=47.36, p=2x10^{-16}) (Table 1.4). K for Chilko and Anderson-Seton fish did not differ, but were lower than the Shuswap and Cultus fish, which also did not differ. Similarly, in 2010, K was significantly lower in Chilko smolts than the Cultus smolts (t=6.98, p=9x10^{-7}). For stocks sampled in multiple years, there were no significant differences in smolt condition factor between years; for Chilko smolts (F_2=2.37, p=0.11) and for Cultus smolts (t=-0.82, p=0.42).

Temporal variation in smolt physiology

Chilko

There was a positive relationship between gill NKA activity and day of collection at the start of migration ($F_{1,44}$=10.66, p=2.12x10^{-3}). Gill NKA was significantly higher on the last sample day (May 16) compared to the first (Apr 21) and third sample day (May 8) (Fig. 1.3). No relationship was found between day of collection and NKA activity for the smolts captured at the Lower River site ($F_{1,16}$=0.94, p=0.35).

Fish ranged in length at the start of migration (F_3=5.92, p=1.72 x10^{-3}); the fish sampled on May 8, the third sample day, were significantly smaller than the fish sampled at the beginning (Apr 21) and end (May 16) (Table 1.5). Fish that left the natal lake on the last sample day (May 16) had higher mean condition factor than fish migrating earlier (F_3=9.65, p=4.9x10^{-5}). Fish sampled at the Lower River site ranged from 63 to 96 mm, however significant differences in length were not observed across day of collection (F_3=2.59, p=0.09).

Shuswap

Shuswap sockeye salmon were sampled over a greater range of dates than Chilko sockeye salmon and showed more temporal variation in NKA activities at all three capture locations (Pre Migration, Migration Start and Lower River) (Fig. 1.4). Fish captured in the lake

before migration showed a significant increase in NKA activity between the two dates (t=8.71, $p=3.22x10^{-11}$). Sampling out migrating smolts on Little River revealed significant changes in NKA activity over time ($F_6=4.92$, $p=2.99 x10^{-4}$). Peak NKA activity was measured for the second sample date (May 10) and then declined; samples collected between May 24 and Jun 14 were significantly lower than the peak. Shuswap smolts sampled at the Lower River site did not differ in NKA activity among sample dates ($F_5=1.65$, $p=0.21$).

There was a significant difference in the length of fish captured pre migration in the natal lake (t=5.66, $p=9.21x10^{-7}$) (Table 1.5). There were also significant differences in the length of fish at the start of migration ($F_6=5.1$, $p=1.87x10^{-4}$), but no pattern was evident. The condition factor of smolts also varied significantly across sample date during the migration start ($F_6=5.28$, $p=1.32x10^{-4}$), but again little pattern was evident over time. The Lower River smolts did not differ for any of the sample days ($F_5=1.43$, $p=0.26$).

Physiological changes with migration distance

Activity of NKA differed with sampling location, but in general there was an increase with migration distance – the lowest enzyme activities were measured in fish captured in their natal lake and the highest levels were measured in smolts captured in the ocean. NKA activity, however, showed considerable differences among regional group during river migration, but also among year.

Upper Fraser

NKA activity generally increased with distance from Chilko Lake (Fig. 1.5). Variation in this general pattern, however, existed among the three years that fish were sampled. In 2010 NKA activity differed among sample locations ($F_2=38.37$ $p=7.84x10^{-12}$). Smolts sampled at the start of migration had high NKA activities that were similar to those from the Lower River site,

though a significant increase was observed in fish sampled in the ocean. Differences were also observed in 2011 (F_2=26.55, p=1.29x10^{-9}). Smolts leaving Chilko Lake, however, had significantly lower gill NKA activity than smolts from the Lower River site, and the Lower River levels were maintained to the fish sampled in the ocean. In 2012 NKA activities also differed by capture location (F_3=55.72, p=4.99x10^{-15}). Fry collected from the lake had lower NKA activity than smolts sampled at the start of migration. The high gill NKA activities of smolts was not seen in Chilko fish caught in the lower Fraser, however a significant increase in NKA activity was observed for fish sampled in the ocean. The differences in NKA activity patterns with migration distance among sample years were due to differences in NKA activity for a given site. Significant differences existed among years for fish sampled leaving Chilko Lake (as described earlier), fish captured in the lower Fraser (F_2=36.5, p=1.16x10^{-9}), and even for fish sampled in the ocean (F_2=14.45, p=3.82x10^{-6}).

Length of fish differed with sample location in 2010 (F_2=68.94, p=2x10^{-16}), 2011 (F_2=77.21, p=2x10^{-16}), and 2012 (F_3=321.1, p=2x10^{-16}). For each year, there was no difference in size between fish leaving the lake and fish caught in the lower Fraser, though fish caught in the ocean were significantly larger (Table 1.6). A similar trend was observed in the K with significant differences measured in 2010 (F_2=53.74, p=1.19x10^{-14}), 2011 (t=10.17, p=7.66x10^{-15}), and 2012 (F_2=79.92, p=4.55x10^{-12}). Compared to the freshwater sample locations, K was consistently higher for fish caught in the ocean. As with NKA, length and K differed among years at the onset of migration (as described earlier). In the Lower River, the length of fish varied across the three years of the study (F_2=18.25, p=2.14x10^{-6}) (Table 1.6). The ocean capture location also differed across the three years of study (F_2=4.71, p=0.01), where length was highest

in 2012. The condition factor was also marginally different across the three years of the study (F_2=3.1, p=0.05).

Thompson

Differences in capture location in the 2012 Thompson fish showed a similar trend to the Upper Fraser fish with a marked increase in NKA activity from pre migration to the ocean (F_3=127.5, p=$2x10^{-16}$) (Fig. 1.6). In 2011, however, Lower River smolts did not differ from Ocean smolts (t=-0.32, p=0.75) (Fig. 1.6). Significant differences in years were observed in the Lower River samples between the 2011 and 2012 samples (t=-6.1, p=$1.06x10^{-6}$) (Fig. 1.6). No difference in NKA levels, however, was observed in the ocean samples across the three years of the study (F_2=2.602, p=0.0785).

Variability in length and condition factor was observed across capture location and year of study for the Thompson fish. Smolts in 2011 at the Lower River capture location were significantly smaller than the fish collected in the ocean (t=3.88, p=$1.21x10^{-3}$). In 2012, length increased from the pre migration smolts to the ocean fish (F_2=152.2, p=$2x10^{-16}$) (Table 1.6). Similarly, the condition factor differed by capture location (F_2=43.52, p=$2.59x10^{-15}$). Significant differences in length were observed in the Lower River samples between the 2011 and 2012 samples (t=-3.67, p=$8.77 x10^{-3}$). As well, fish length differed across the three years of the study in the ocean samples (F_2=31.6, p=$1.5x10^{-11}$). Differences in condition factor, however, were not observed in the ocean samples across the three years of the study.

Lower Mid-Fraser

Differences in capture location in the Lower Mid Fraser showed an increase in gill NKA activity from the start of migration to fish caught in the ocean (Fig. 1.7). In, 2012, an increase was observed in the smolts from the start of migration to the Lower River smolts (t=2.81,

p=0.02). This trend of increasing NKA levels was observed for all the ocean samples (mean across 2010-2012) (F_2=31.61, p=6.48x10^{-10}).

The length of smolts from the Lower Mid-Fraser group differed by smolt location (F_2=170.3, p=2x10^{-16}), and the increase was observed after seawater entry (Table 1.6). As well, the condition factor differed between the smolts at the start of migration and in the ocean (t=10.39, p=1.86x10^{-11}).

Lower Fraser

In 2010, differences in capture location in the Lower Fraser sockeye salmon showed an increase from the pre-migration smolts to the ocean fish (F_3=35.8, p=6.69x10^{-11}) (Fig. 1.8). At the pre-migration location, there was no difference between the 2010 and 2011 fish sampled (t=-1.03, p=0.32). Gill NKA activity for smolts at the migration start, however, differed significantly (as described earlier), but the highest enzyme activities were measured in the ocean.

There were significant differences in the length of fish at the different capture location in 2010 (F_3=7.77, p=1.78x10^{-9}) (Table 1.6). As well differences were observed for the condition factor of fish at the different capture locations (F_3=8.71, p=1.77x10^{-4}). Pre migration fish differed in length and K due to the time of year when the fish were sampled (t=-5.54, p=1.45x10^{-5}; and t=-2.88, p=8.57x10^{-3} for length and K, respectively). Differences in years for length and condition factor of smolts were also found at the start of migration (as described above).

Table 1.4: Length (L) and condition factor (K) (means ± SE) for migrating sockeye salmon intercepted below the natal lake for four stocks from the Fraser River watershed. Fish were sampled near the peak of downstream migration. Significant differences are shown for values that do not have a common letter ($p < 0.05$); upper case letters for comparisons among stock for the same year and lower case letters for comparison within stock over different years. Sample dates are shown in Table 1.1. Data are presented as means ± SE.

Stock	Capture Location	Year					
		2010		2011		2012	
		L ± se	K ± se	L ± se	K ± se	L ± se	K ± se
Chilko	Chilko River	95.8 ± 4.9^{Aa}	0.75 ± 0.01^{Ba}	89.2 ± 2.3^{a}	0.74 ± 0.01^{a}	76.5 ± 1.2^{Bb}	0.72 ± 0.01^{Ba}
Shuswap	Little River					67.7 ± 2.2^{C}	0.84 ± 0.02^{A}
Anderson-Seton	Seton River					76.6 ± 0.7^{B}	0.71 ± 0.01^{B}
Cultus	Sweltzer Creek	104.9 ± 1.5^{Aa}	0.89 ± 0.02^{Aa}			92.9 ± 2.0^{Ab}	0.87 ± 0.02^{Aa}

Table 1.5: Mean length (L) and condition factor (K) (±SE) of sockeye salmon for Chilko and Shuswap stocks in the Fraser River watershed caught approximately weekly at the same location; pre-migration within the natal lake, at the mouth of the river when the fish were starting migration, and before migration into the ocean in the Lower River. NA indicates weight observations were not taken on samples. Significant differences are shown for values that do not have a common letter ($p < 0.05$). Sample dates are shown in Table 1.2. Data are presented as means ± SE.

Stock	Capture Location					
	Lake (Pre Migration)		Migration Start		Lower River	
	L ± se	K ± se	L ± se	K ± se	L ± se	K ± se
Chilko			79.1 ± 1.1^{A}	0.72 ± 0.02^{B}	78.8 ± 1.8^{A}	NA
			76.5 ± 1.2^{BA}	0.72 ± 0.01^{B}	81.6 ± 3.0^{A}	NA
			72.2 ± 2.3^{B}	0.72 ± 0.01^{B}	67.5 ± 4.5^{A}	NA
			80.6 ± 1.1^{A}	0.81 ± 0.02^{A}	74.0^{A}	NA
Shuswap	61.0 ± 1.0^{B}	0.68±0.01	69.1 ± 2.7^{BC}	0.79 ± 0.02^{B}	68.5 ± 1.5^{A}	NA
	71.0 ± 1.6^{A}	NA	74.3 ± 1.9^{ABC}	0.94 ± 0.03^{A}	73.7 ± 2.0^{A}	NA
			70.2 ± 2.4^{BC}	0.87 ± 0.02^{AB}	69.3 ± 3.0^{A}	NA
			67.7 ± 2.2^{BC}	0.84 ± 0.02^{B}	76.0^{A}	NA
			65.1 ± 2.8^{C}	0.83 ± 0.02^{B}	71.1 ± 1.1^{A}	NA
			80.6 ± 1.8^{A}	0.81 ± 0.02^{B}	79.0^{A}	NA
			75.2 ± 2.3^{AB}	0.88 ± 0.02^{AB}		

Table 1.6: Mean length (L) and condition factor (K) (±SE) for sockeye salmon intercepted at different locations during their migration in the Fraser River watershed and after ocean entry. NA indicates weight observations were not taken on samples. Significant differences are shown for values that do not have a common letter ($p < 0.05$); upper case letters for comparisons among groups for the same year and lower case letters for comparison within groups over different years. Sample dates are shown in Table 1.3. Data are presented as means ± SE.

Region	Capture location							
	Pre Migration		Migration start		Lower river		Ocean	
	L ± se	K ± se	L ± se	K ± se	L ± se	K ± se	L ± se	K ± se
Upper Fraser								
	Chilko Lake		*Chilko River*		*Fraser River*		*Strait of Georgia*	
2010			95.8 ± 4.9^{Ba}	0.75 ± 0.01^{Ba}	90.3 ± 2.1^{Ba}	0.77 ± 0.01^{B}	120.5 ± 1.2^{Ab}	0.92 ± 0.01^{Aab}
2011			89.2 ± 2.3^{Ba}	0.74 ± 0.01^{Ba}	94.6 ± 2.1^{Ba}	NA	120.8 ± 1.7^{Ab}	0.93 ± 0.01^{Aa}
2012	29.2 ± 0.4^{C}	0.58 ± 0.02^{C}	76.5 ± 1.2^{Bb}	0.72 ± 0.01^{Ba}	78.5 ± 1.7^{Bb}	NA	131.9 ± 4.4^{Aa}	0.85 ± 0.02^{Ab}
Thompson								
	Shuswap Lake		*Little River*		*Fraser River*		*Strait of Georgia*	
2010							118.2 ± 3.1^{b}	0.94 ± 0.05^{a}
2011					94.2 ± 1.2^{Ba}	NA	136.4 ± 4.4^{Aa}	0.86 ± 0.06^{a}
2012	61.0 ± 1.0^{C}	0.68 ± 0.01^{B}	67.7 ± 1.2^{BC}	0.84 ± 0.02^{A}	71.5 ± 0.9^{Bb}	NA	102.4 ± 1.3^{Ac}	0.91 ± 0.01^{Aa}
Lower Mid-Fraser								
	Anderson Lake		*Seton River*		*Fraser River*		*Strait of Georgia*	
2010							119.0	0.89
2011							118.0	0.96
2012			76.6 ± 0.7^{B}	0.71 ± 0.01^{B}	78.4 ± 0.8^{B}	NA	125.0	0.87
					2010-2012 Ocean pooled		*120.7±2.2^A*	*0.91±0.03^A*
Lower Fraser								
	Cultus Lake		*Sweltzer Creek*		*Fraser River*		*Strait of Georgia*	
2010	93.3 ± 1.4^{Ca}	0.96 ± 0.02^{Aa}	104.9 ± 1.5^{Ba}	0.89 ± 0.02^{Aa}	85.9 ± 2.4^{C}	0.77 ± 0.01^{B}	116.1 ± 3.8^{A}	0.85 ± 0.04^{B}
2011	80.4 ± 1.9^{b}	0.88 ± 0.02^{b}					134.0	0.85
2012			92.9 ± 2.0^{b}	0.87 ± 0.02^{a}				

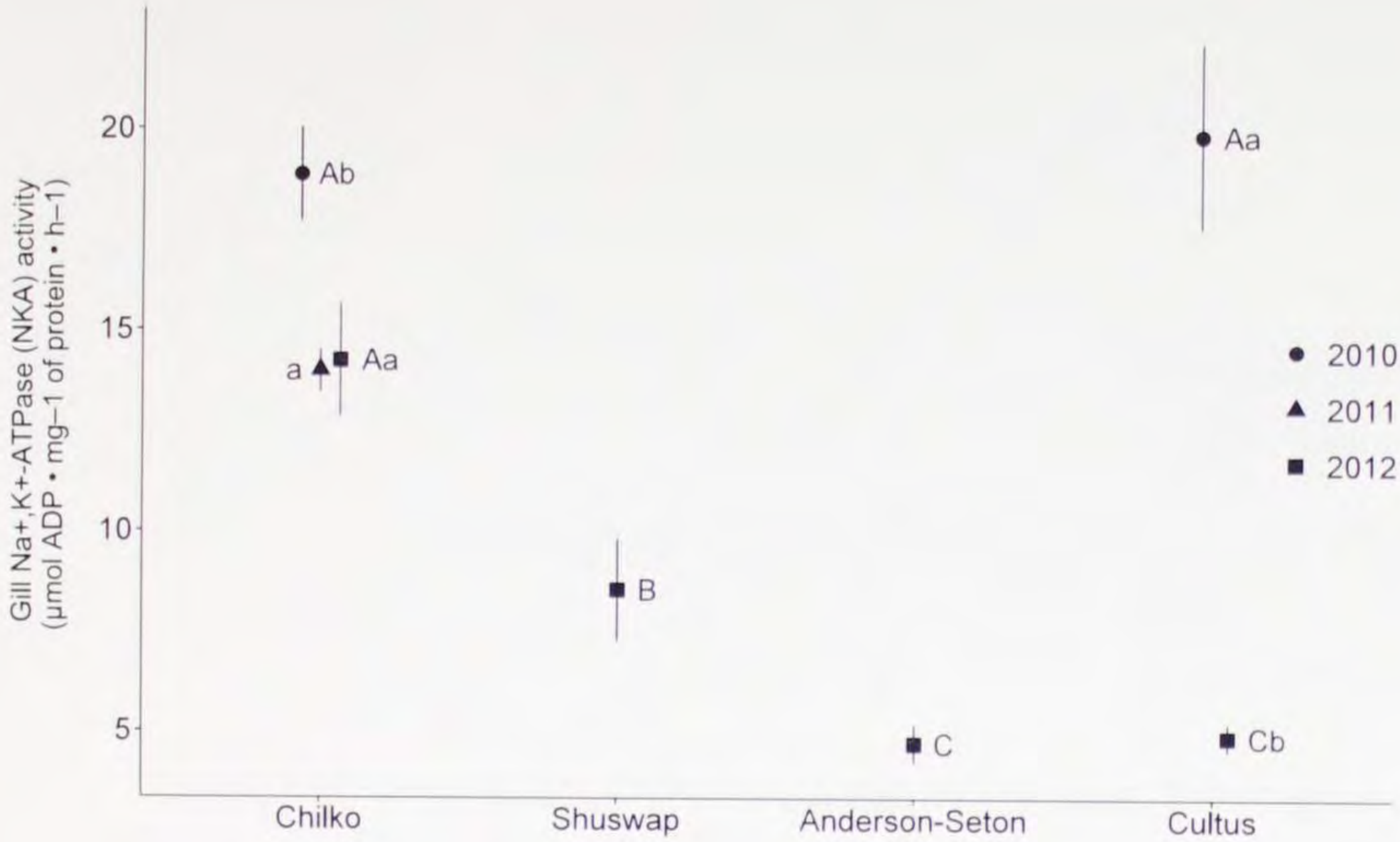

Figure 1.2: Gill Na^+/K^+-ATPase (NKA) activity (µmol ADP • mg^{-1} of protein • h^{-1}) from four stocks in the Fraser River Watershed in 2010 (circles), 2011 (triangles) and 2012 (squares). Significant differences are shown for values that do not have a common letter ($p < 0.05$); upper case letters for comparisons among groups for the same year and lower case letters for comparison within groups over different years. Data are presented as means ± SE.

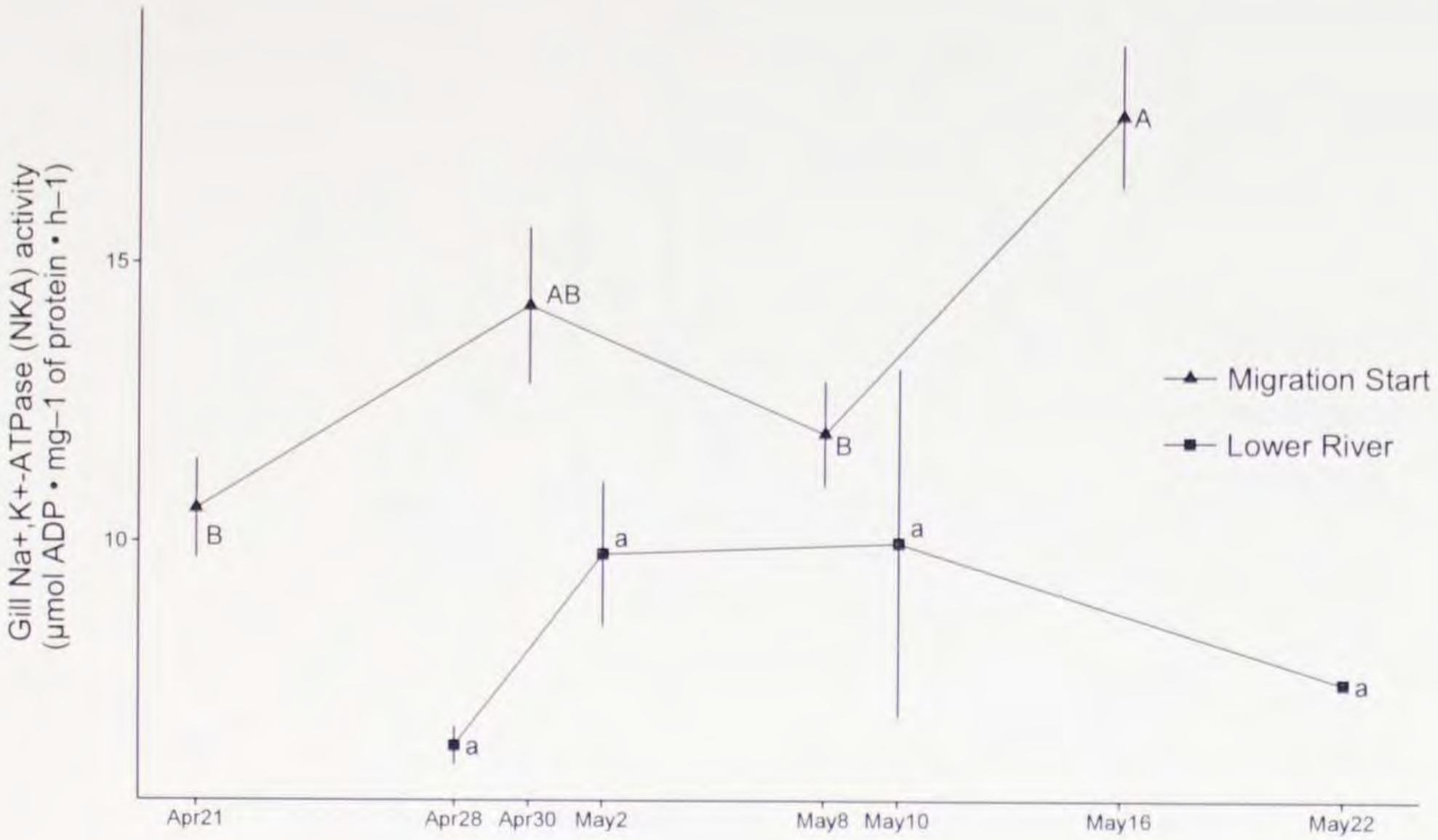

Figure 1.3: Chilko Gill Na^+/K^+-ATPase (NKA) activity (µmol ADP • mg^{-1} of protein • h^{-1}) from April 21, 2012 to May 22, 2012 from the start of migration at Chilko River (triangles), and in the Lower River at Mission (squares). Significant differences are shown for values that do not have a common letter ($p < 0.05$); comparisons with collection day are denoted as upper case letters for Migration Start, and lower case letters for the Lower River fish. Data are presented as means ± SE.

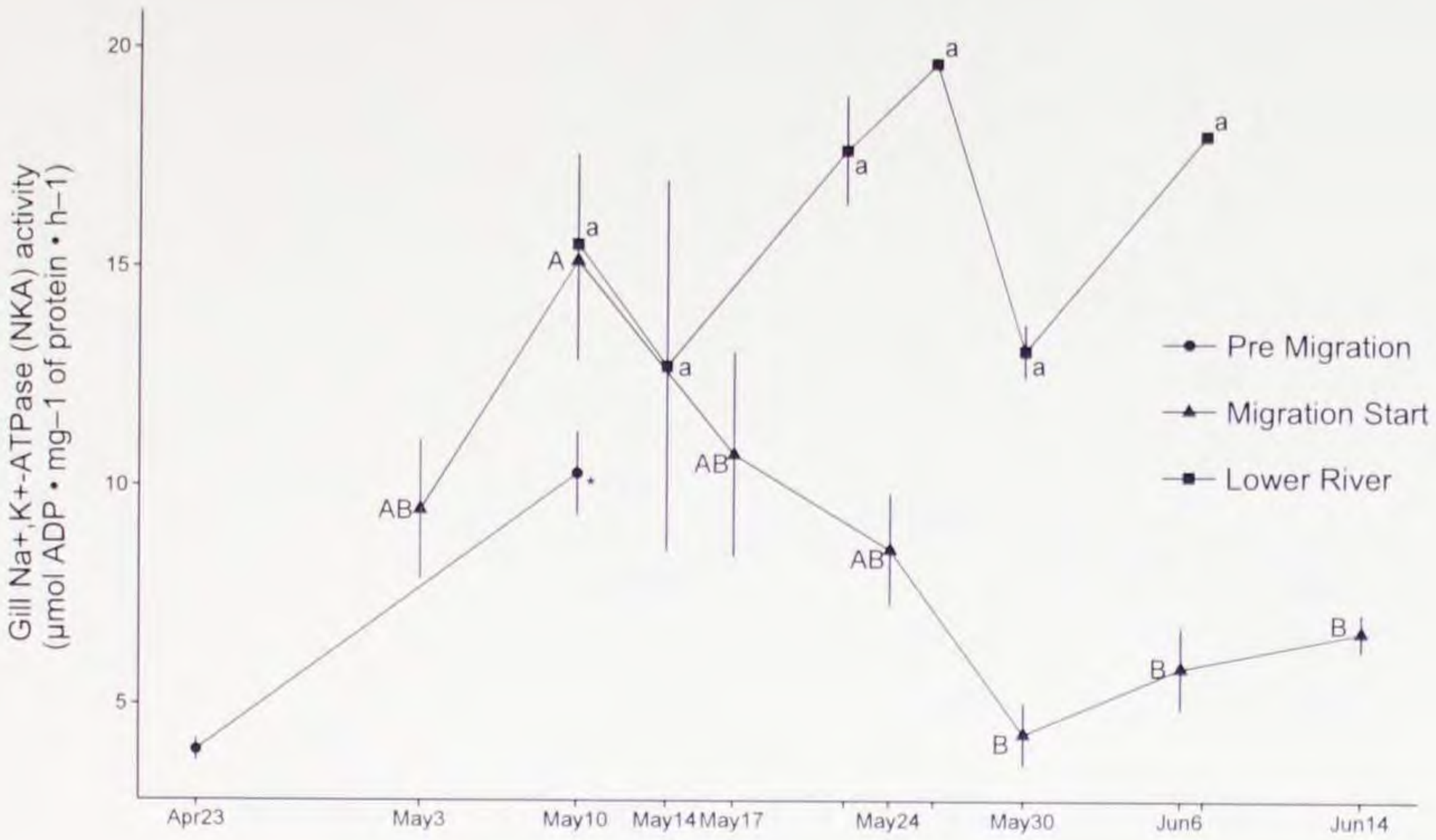

Figure 1.4: Gill Na^+/K^+-ATPase (NKA) activity (µmol ADP • mg^{-1} of protein • h^{-1}) from April 23, 2012 (Day 114) to June 14, 2012 (Day 166) for Shuswap Lake juvenile sockeye salmon. Fish captured pre migration in Shuswap Lake (circles), at the start of migration at Little River (triangles) and in the Lower River at Mission (squares). Significant differences are shown for values that do not have a common letter or symbol ($p < 0.05$); comparisons with collection day are denoted as significantly different with an * for the Pre Migration Fish, as upper case letters for Migration Start, and lower case letters for the Lower River fish. Data are presented as means ± SE.

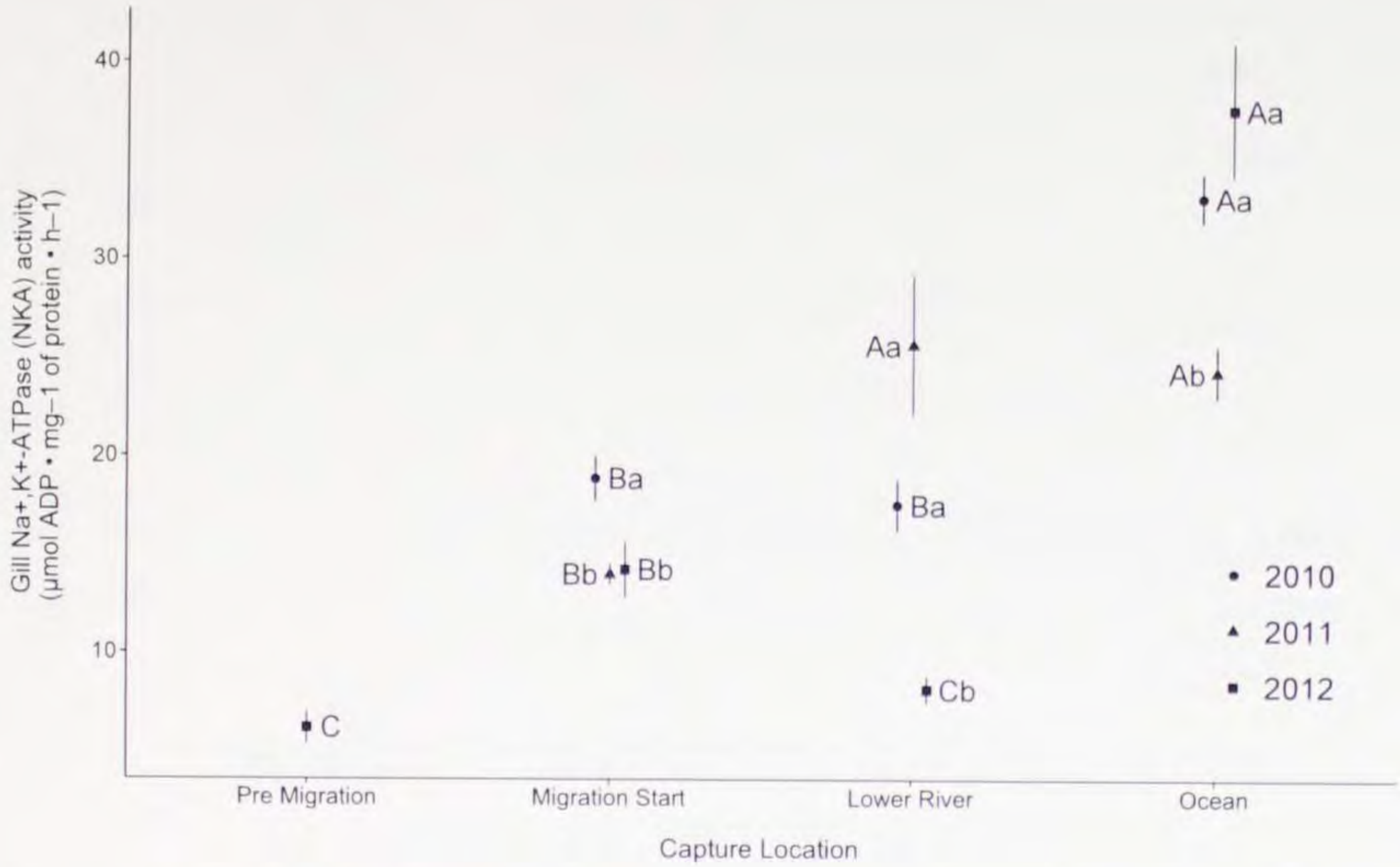

Figure 1.5: Gill Na^+/K^+-ATPase (NKA) activity (µmol ADP • mg^{-1} of protein • h^{-1}) of Upper Fraser juvenile sockeye salmon smolts intercepted at different locations during their migration for in the Fraser River watershed and after ocean entry in the Fraser River watershed and after ocean entry in 2010 (circles), 2011 (triangles) and 2012 (squares). Significant differences are shown for values that do not have a common letter ($p < 0.05$); upper case letters for comparisons between capture location for the same year and lower case letters for comparison within capture location over different years. Data are presented as means ± SE.

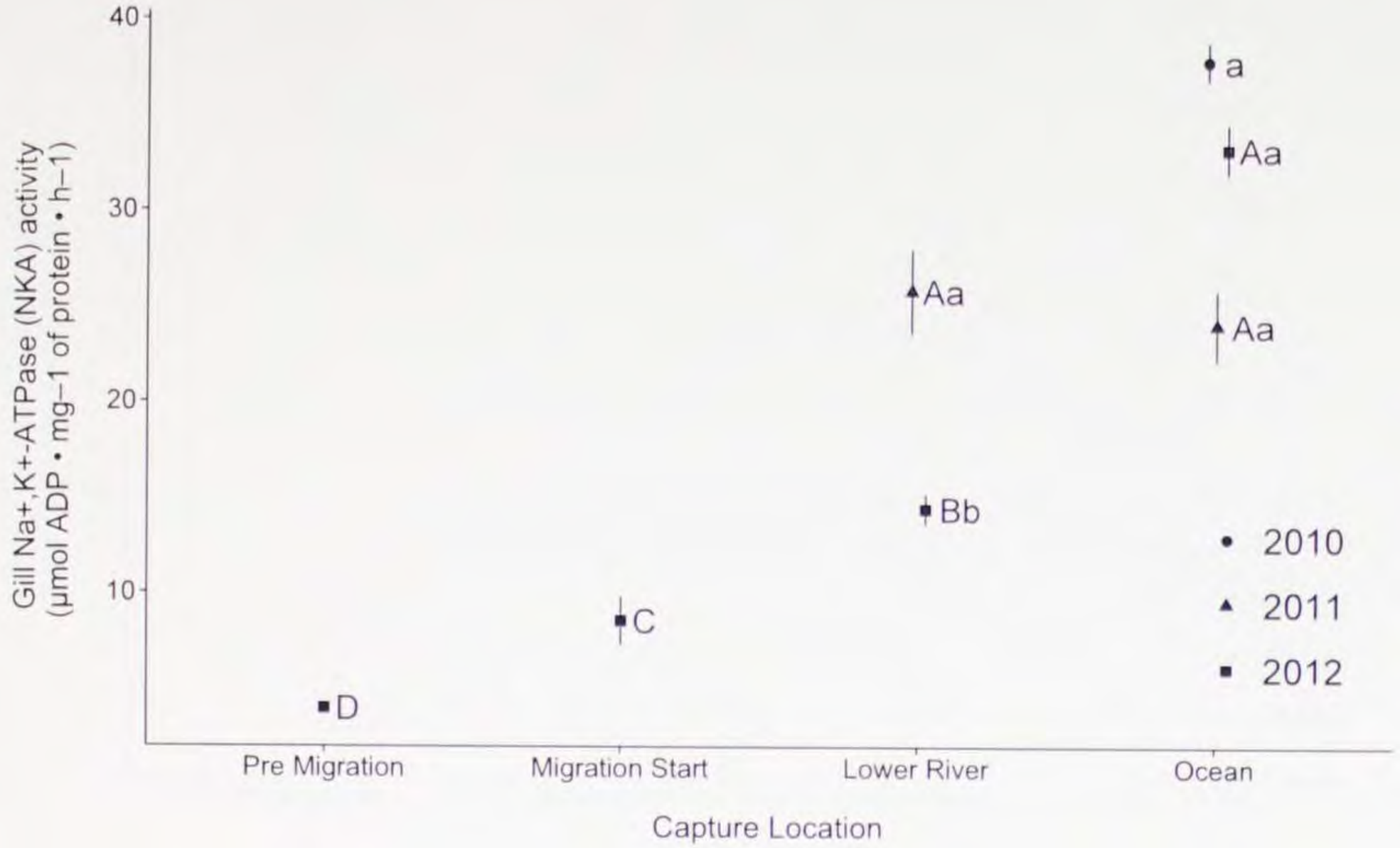

Figure 1.6: Gill Na^+/K^+-ATPase (NKA) activity (µmol ADP • mg^{-1} of protein • h^{-1}) of Thompson juvenile sockeye salmon smolts intercepted at different locations during their migration for in the Fraser River watershed and after ocean entry in the Fraser River watershed and after ocean entry in 2010 (circles), 2011 (triangles) and 2012 (squares). Significant differences are shown for values that do not have a common letter ($p < 0.05$); upper case letters for comparisons between capture location for the same year and lower case letters for comparison within capture location over different years. Data are presented as means ± SE.

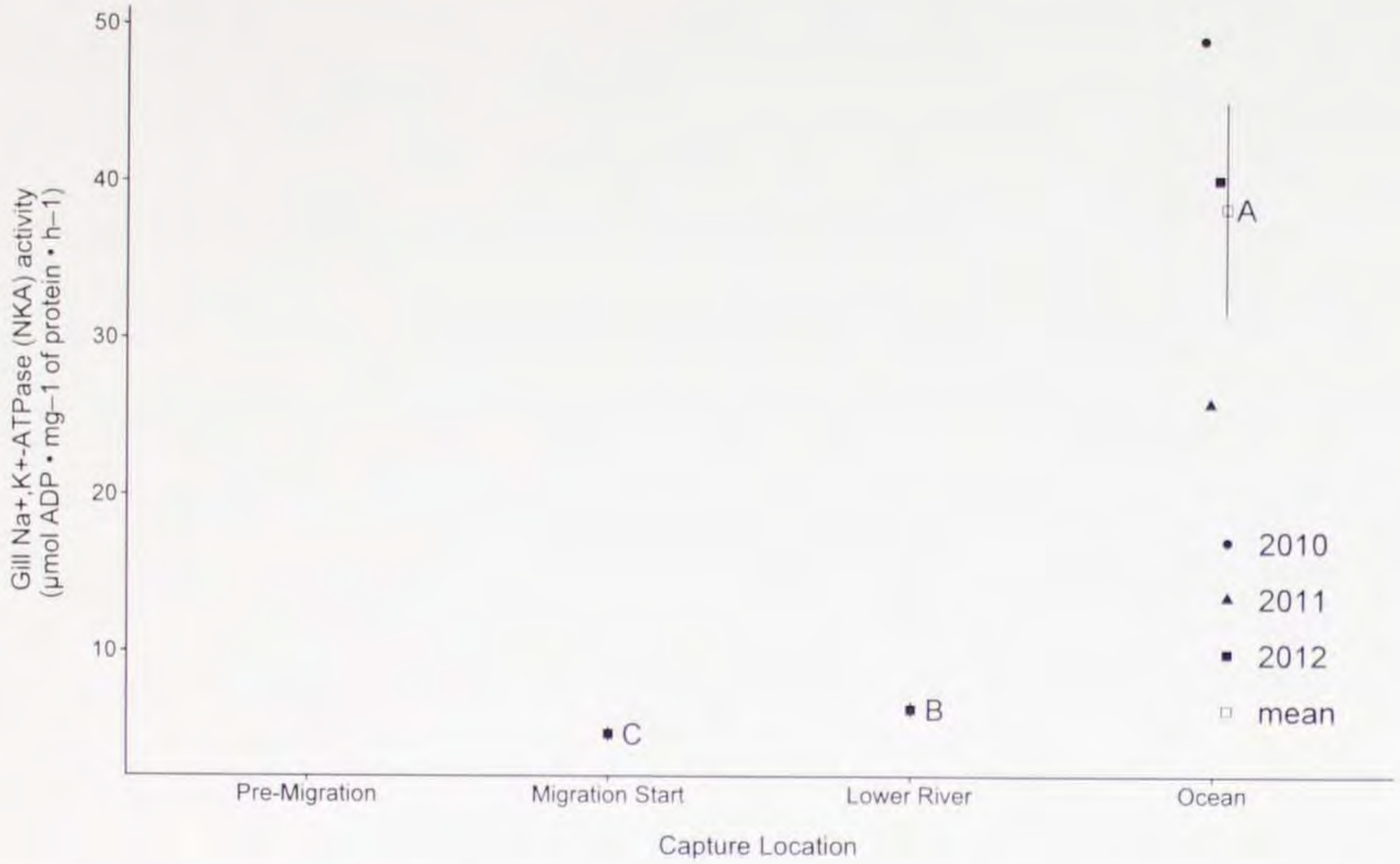

Figure 1.7: Gill $N+^{+}/K^{+}$-ATPase (NKA) activity (µmol ADP • mg^{-1} of protein • h^{-1}) of Lower Mid-Fraser juvenile sockeye salmon smolts intercepted at different locations during their migration for in the Fraser River watershed and after ocean entry in the Fraser River watershed and after ocean entry in 2010 (circle), 2011 (triangle), 2012 (squares) and mean across three years of study for ocean samples (open square). Significant differences are shown for values that do not have a common letter ($p < 0.05$); upper case letters for comparisons between capture location for the same year and lower case letters for comparison within capture location over different years. Data are presented as means ± SE.

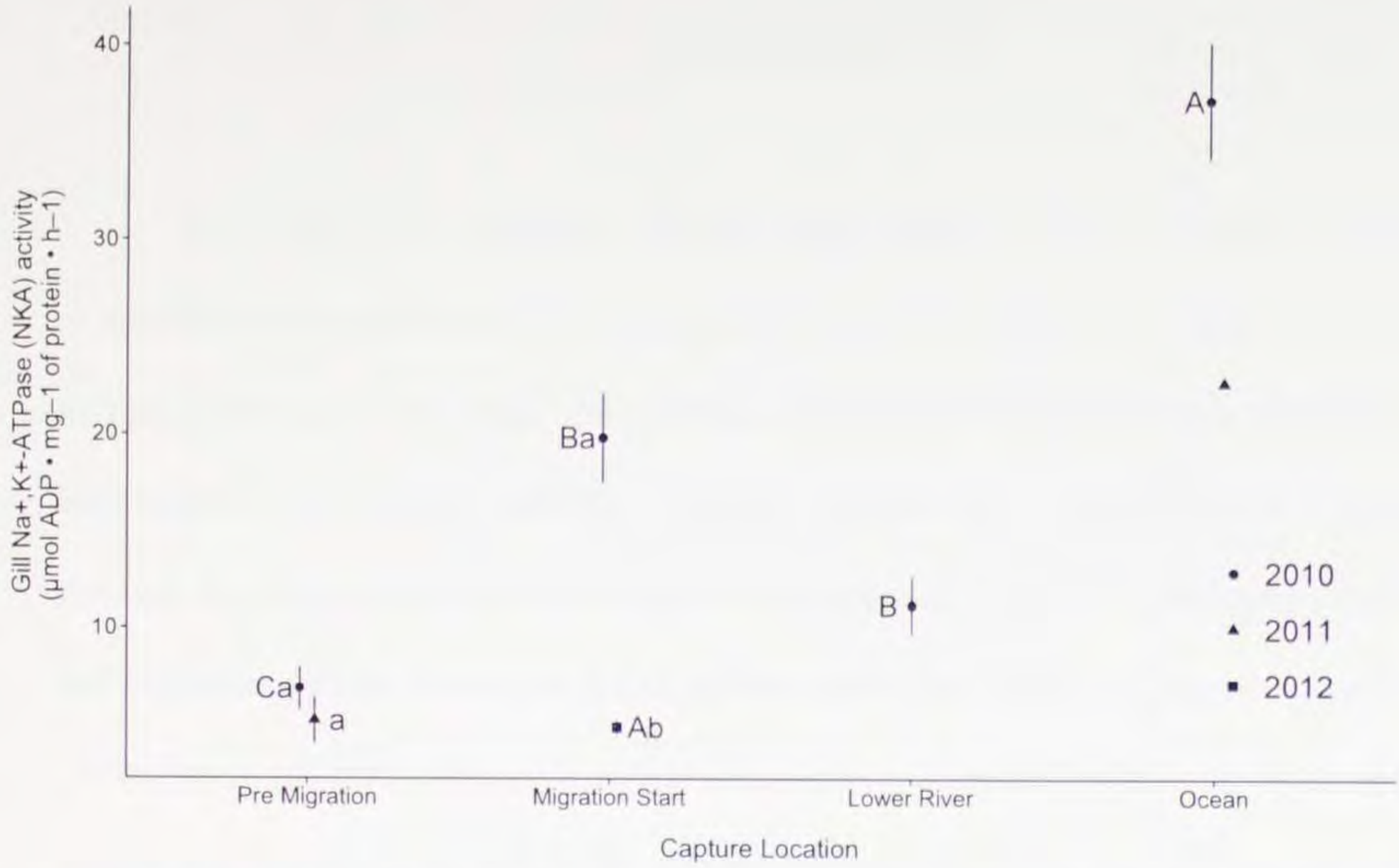

Figure 1.8: Gill Na^+/K^+-ATPase (NKA) activity (µmol ADP • mg^{-1} of protein • h^{-1}) of Lower Fraser juvenile sockeye salmon smolts intercepted at different locations during their migration for in the Fraser River watershed and after ocean entry in the Fraser River watershed and after ocean entry in 2010 (circles), 2011 (triangles) and 2012 (squares). Significant differences are shown for values that do not have a common letter ($p < 0.05$); upper case letters for comparisons between capture location for the same year and lower case letters for comparison within capture location over different years. Data are presented as means ± SE.

DISCUSSION

The Fraser River watershed offered an opportunity to compare among populations of sockeye salmon and assess how smolt physiology was affected by location of natal rearing environment. My study showed that at the start of migration, NKA activity levels were high for long distance migrators and more variable for the short distance migrators. As well, my study demonstrated that there was temporal variation in NKA activity during the migration season. Juvenile sockeye salmon intercepted during migration also showed variability in gill NKA activity, however substantially higher NKA activity levels were observed in seawater. The spatial and temporal variability observed in my results were likely driven by local adaptation for the natal lake systems, but also environmental differences experienced during downstream migration.

Is there a population difference in NKA activity in smolts at the start of migration?

At the onset of migration, fish from all populations left their natal lake with gill NKA activity levels that were characteristic of competent smolts. However, the highest gill NKA levels were consistently observed for the long distance migrators, and more variable enzyme activities for the short distance migrators (Fig. 1.2). In 2010, there was no difference in gill NKA activity between the Chilko smolts, (which have 684 km to migration to seawater) and the Cultus smolts (which have 113 km to migrate). In 2012, however, there was a positive relationship between gill NKA activity and distance of the natal lake to the ocean. Only Chilko Lake smolts were intercepted in 2011, but gill NKA was also high for these fish. A positive relationship or lack of a relationship between migration distance and gill NKA activity at the start of migration was surprising. Sockeye

salmon smolts that left Chilko Lake appeared to be competent smolts even though entry into seawater did not occur for approximately one week (Rechisky et al., 2013). In contrast, sockeye salmon smolts from the lower Fraser River populations were likely to enter the ocean within days after they left the lake – but some had lower gill NKA activities. With the short migration distance to the ocean, gill NKA activity needed to increase significantly before seawater entry. Gill NKA activity has been shown to increase in approximately 7 days following hormone treatment (Shrimpton and McCormick, 1998) or up to 14 days following transfer to seawater (Flores and Shrimpton, 2012). Rapid activation of gill NKA activity, however, occurs within days for rainbow trout (*O. mykiss*) after direct transfer to seawater during the spring (Madsen and Naamansen, 1989) – suggesting that juvenile sockeye salmon may be able to upregulate gill NKA activity rapidly before seawater entry during the smolt window. Conversely, the high gill NKA activities for the long distance migrating smolts allowed for a decrease to occur, but for activities to remain within the range typical for the smolt window. The physiological benefit of such high gill NKA at the time of migration start is unclear.

Differences in gill NKA were also seen among years. Reasons for the differences may be related to growth opportunities. Evaluation of juvenile coho salmon smolting demonstrated that large fish exhibit higher gill NKA activity (Shrimpton, 1996). In my study, however, Chilko Lake smolts were smaller than Cultus Lake smolts. Shrimpton et al. (1994) found that larger hatchery fish had reduced saltwater tolerance, and lower NKA gill activity than to their wild counterparts indicating that environment influenced smolting. Whether the lake environments in this study represented enough of a difference to account for the results in not clear, but growth opportunities may be revealed in

condition factor. A decrease in condition factor is a characteristic of smolting, from the energetic demands associated with smolting (McCormick and Saunders, 1987) or loss of total body lipids (Wedemeyer et al., 1980). However, at the onset of migration, sockeye salmon smolts in my study although different in size, had similar condition factors.

Is there a temporal difference in NKA activity at any specific stage of migration?

Gill NKA activities showed significant differences for smolts leaving the lake for the Chilko and Shuswap populations. Gill NKA activity for Chilko smolts intercepted as they left the lake, increased over time and was highest on the last date sampled. Similarly, gill NKA activity levels for Shuswap smolts differed at the start of migration. Lowest enzyme activities were for early and late migrants in the run, where the NKA activity levels peaked on May 10. The peak gill NKA activities for Chilko smolts on May 18 may have been followed by decreasing activities, but could not be measured due to cessation of sampling occurs with operations to remove the fence prior to high Chilko River flows. Smolts captured in the Lower River showed less temporal variation and the differences were not significant.

Variables such as photoperiod, temperature, flow and energetics (Hoar, 1988) have previously been shown to influence smolt physiology. Seasonal differences of these variables may account for the variability observed in gill NKA activity at the start of migration. Gill NKA activity increased seasonally with longer day-length in the spring in hatchery-reared Atlantic salmon smolts (Shrimpton et al., 2000). The temporal pattern characterized for these smolts was similar to that observed for wild smolts emigrating from Shuswap Lake and the increase with time observed for Chilko Lake. My results also demonstrated that sockeye salmon smolts were not initiating migration with peak gill

NKA activity. For Atlantic salmon maintained on a simulated natural photoperiod, both behavioural (downstream movements) and physiological (NKA activity) changes were dependent on cumulative thermal experience rather than a threshold temperature (Zydlewski et al., 2005). Similarly, cumulative thermal experience influenced migration timing in Chinook salmon smolts (Sykes et al., 2009). However, some research suggests a threshold temperature initiates smolting. An accelerated temperature regime advanced the timing of peak NKA activity of Atlantic salmon smolts (McCormick et al., 2002). Clarke et al. (1978) found this threshold to be 17.5 °C for sockeye salmon. Sockeye salmon smolts at Chilko Lake were initiating migration in the spring at mean daily temperatures of approximately 4-6 °C, much lower than threshold temperatures reported in the literature. As temperatures are so much lower than thresholds previously described cumulative thermal experience may be important for Fraser River sockeye salmon smolt development. Additionally, sockeye salmon are not experiencing dramatic temperature fluctuations in their natal lakes. Lake temperatures fluctuate minimally in the spring. Consequently, migrating smolts are not likely to experience much change in temperature until they are downstream of the lake and well underway in their migration.

Water current has also been shown to influence migratory behaviour. In response to a strong, directional current, Chinook salmon smolts adjusted behaviour to a period of defined movement (Sykes and Shrimpton, 2010). Linking flow to successful survival of smolts was addressed when environmental factors were tested against freshwater survival and production of coho salmon in Oregon (Lawsen et al., 2004). Flow variables, such as second winter flows, date of first fall freshet and flow during outmigration, were all correlated strongly to smolt production. This suggests there are environmental advantages

associated with an increase in flow. Similar to the influence on pre migration temperature, the changes in flow prior to migration would not be appreciable for fish rearing in natal lakes. For sockeye salmon smolts, higher flow during migration coupled with active swimming in smolts will decrease the time needed to migrate, making saltwater entry during the smolt window more likely.

Energetic reserves and feeding opportunities may also affect temporal variation in smolt physiology. For the Chilko smolts, the high gill NKA activities at the last sample date (Fig. 1.3) corresponded with increased condition factor. Perhaps, later departure from the natal lake allowed for more time to feed before migration. In contrast, later departure does not appear to benefit condition factor for the Shuswap smolts; condition factor of smolts was highest on the second sample date and coincident with the peak in gill NKA activity. Condition factor was also high on the last sample date for Shuswap smolts when gill NKA activities were significantly lower than the peak.

Does migration affect smolt characteristics?

Smolt physiology was dynamic during migration and post seawater entry. Gill NKA activity was lowest for fish that were sampled pre migration and highest for fish in the ocean, but the pattern of increase in gill NKA varied among stocks and years. In general, there were three patterns for smolting characteristics observed; gill NKA was high at the start of migration and stayed high, gill NKA was low at the start of migration and increased, and gill NKA was variable throughout migration.

Gill NKA activity measurements suggest that development of saltwater tolerance was coincident with initiation of downstream migration for the Upper Fraser fish in 2010. This pattern of development would suggest that behavioural and physiological smolt

characteristics develop synchronously at the start of migration and change little until seawater entry. The seasonal changes in environmental cues that stimulated smolting appeared to trigger both the behaviour of migration and the physiological development of seawater tolerance. There is considerable evidence that the springtime increases in photoperiod and temperature drive smolt development in other species of salmonids. For example, juvenile Atlantic salmon smolts reared in early spring with increasing photoperiod and temperature exhibited significantly greater gill NKA activity than treatments where photoperiod did not increase (McCormick et al., 2002) or temperatures were cold (McCormick et al., 2000). This pattern suggests a simple response to seasonal changes in environmental that result in synchronous development of physiology and behaviour in smolts.

An increase in gill NKA and smolt development was also observed during downstream migration for the Upper Fraser fish in 2011, Thompson fish in 2012, and in Lower Mid-Fraser fish in 2012. This pattern of development has also been found in hatchery-reared Chinook smolts. Fish intercepted during migration had higher levels of gill NKA activity than those measured at the time of release from the hatchery (Muir et al., 1994) suggesting that current and swimming positively affect smolt development. Current has also been shown to synchronize swimming behaviour in hatchery Chinook smolts; however, increased current speed and swimming had no effect on gill NKA activity (Sykes and Shrimpton, 2010). Whether migration will augment smolt physiology in wild fish is not known, but smolt development differs considerably between hatchery-reared and wild smolts (Shrimpton et al., 1994). There is also evidence that migration may not be linked to smolt physiology as downstream movement has been documented to

occur prior to the parr-smolt transformation (Shrimpton et al., 2014). This is the first evidence of smolt physiology changing in sockeye salmon following emigration from their natal lake – suggesting that behaviour may be uncoupled from physiology in smolting.

The first two patterns of changes in gill NKA activity appear to be mutually exclusive, but may actually fit within the third potential pattern: smolt physiology may be highly dynamic until marine entry. Behaviour and smolting are likely coupled at the onset of migration, however, adjustments may be continuously occurring during migration in response to environmental cues. Increases in temperature both increase (McCormick et al., 2000) and decrease (McCormick et al., 1997) gill NKA activity. Increased springtime flows are also linked with higher suspended sediment levels that inhibit smolt development (Shrimpton et al., 2007). A decline in gill NKA activity has also been recorded in Atlantic salmon smolts when fasted in seawater (McCormick, unpublished data), however, this was not seen in a similar experiment on coho salmon smolts (Shrimpton et al., 1994). Whether sockeye salmon smolts are feeding while migrating downstream was not clear as some populations sampled in the lower parts of the river were larger and had higher condition factors, while others did not differ in size and had lower condition factor – but no clear pattern was observed. Changes in external variables during migration may drive the fluctuations in gill NKA activity and maintenance of internal homeostasis may also require physiological adjustments. High gill NKA activities and morphological changes in the gill during the spring when smolts were still in fresh water have been linked to decreases in plasma ion levels (Shrimpton et al., 1994) – but such perturbations in plasma ions may decrease swimming performance (Brauner et

al., 1992). Consequently, smolts may continuously adjust gill ionocyte physiology, including NKA activity, to maintain homeostasis prior to seawater entry. A dynamic relationship may better describe the varied results in this study, where patterns were neither spatially nor temporally explained. Zydlewski and Zydlewski (2011) demonstrated that gill NKA activity was highly variable in smolts prior to seawater entry, yet seawater tolerance and long term growth was not related to prior gill NKA activity – aligning with the theory that smolting is dynamic and individual fish make continual adjustments to physiological function during migration.

Significant changes occurred in gill NKA activity during migration, however similar trends were not observed in length and condition factor – which is not surprising given the short time period for migration. The exception is the Lower Fraser fish; fish collected at Cultus had significantly higher gill NKA activities, were larger and had higher condition factor than the Lower River fish. Fish captured in the Lower River, however, were not from the Cultus stock, but from the Chilliwack Lake population. Size differences observed for the Cultus smolts were likely due to the fact that the productivity in Cultus is high, and as a result, growth opportunities are expected to be much better (Northcote and Larkin, 1956). Overall, length and condition factor was not strongly associated with the gill NKA activity levels, suggesting growth is not driving the changes in physiological smolt characteristics.

Fish sampled in the ocean generally had the highest gill NKA activities, were significantly larger, and exhibited some of the highest values for condition factor. These measures suggest sockeye salmon smolts sampled have made a successful transition to seawater. High gill NKA activity immediately prior to seawater entry for survival,

however, may not be necessary. It is generally accepted that NKA activity increases with smolts as they become tolerant to seawater (McCormick and Saunders, 1987). Atlantic salmon and rainbow trout transferred to seawater exhibit a significant increase in gill NKA activity (McCormick et al., 2008; Flores and Shrimpton, 2012), but the increase took up to 14 days. Smolts will stage in the estuary presumably to adjust to the higher salinities, but Atlantic salmon (McCormick et al., 1998), steelhead and coho salmon (Clements et al., 2012) smolt movements appear more passive and the animals move with the tide. Upregulation of hypo-osmoregulatory ability during the smolt window, however, has been shown to be very rapid (Madsen and Naamansen, 1989). Sockeye salmon smolts, therefore, may not need to reside for very long in the estuary, therefore, and adjust to the increased salinity rapidly.

Conclusion

My study showed the spatial and temporal variation in smolting observed in wild fish. The variation observed in this work illustrates that smolting is a dynamic process. Smolting in anadromous salmon has traditionally been viewed as a cumulative increase in osmoregulatory competence that is preparatory for ocean entry – if fish are unable to successfully enter the marine environment a reversion to the freshwater parr occurs (Hoar, 1988; McCormick et al., 1997; 1999; Shrimpton et al., 2000). This model has been developed from work on multiple species of anadromous salmonids. The work, however, has primarily been derived from studies on fish raised in aquaculture conditions and / or from populations of salmon that have short-distance migrations to reach the ocean. The work on sockeye salmon smolts from the Fraser River watershed, therefore, represents a novel study as wild fish were intercepted at different times during the migration and from

multiple populations where migration distance differed. The model developed from my study suggests that smolting may not be a continual cumulative increase in osmoregulatory competence, but much more dynamic with fluctuations in osmoregulatory enzyme function throughout the smolt window. In general, parr or pre-migratory smolts have low levels of gill NKA activity, smolts in the ocean have high levels of gill NKA activity, and considerable variation exists for fish migrating in the points between. Internal and external factors may influence this variation, but the dynamic nature of smolting is not based on the region of origin, or on the year of migration.

CHAPTER 2

The effect of water temperature on the smolt window for sockeye salmon (*Oncorhynchus nerka*) smolts in the Fraser watershed.

ABSTRACT

The period of time in the spring when behavioural and physiological changes occur to prepare juvenile anadromous salmon for seawater entry is known as the smolt window. Environmental factors such a temperature, however, have previously been shown to influence the duration of the smolt window. In this study, I tested the effect of warm temperature on the length of the smolt window for migrating sockeye salmon smolts on the Chilko River in central British Columbia, Canada. Smolts were held in ambient river water temperature or one of three warmer water treatments. Fish were held for a total of 27 days, and sampled every 4 days. Gill NKA activity remained high throughout the study except for the warmest treatment; the decrease appeared to be a result of exceeding a threshold temperature rather than in response to accumulated thermal units (ATU). Expression of gill NKA α1 isoforms were quantified and the α1a isoform increased from the onset of the experiment and the α1b decreased, consistent with an ATU response. Endocrine results suggest that the stimulus for seawater preparedness had occurred before the fish were captured. At the start of migration, therefore, juvenile sockeye salmon were competent smolts, suggesting the physiological and behavioural aspects of smolting coincide. This research shows that the smolt window is limited and can be abbreviated by high temperatures.

INTRODUCTION

Sockeye salmon, the anadromous form of *Oncorhynchus nerka*, have a life cycle that involves movement between freshwater and marine habitats. Juveniles move from fresh water to seawater after 1-2 years of lake residence. Transitioning to survive in the ocean requires a complex suite of adaptations that occur while fish are still in fresh water, but is preparatory and necessary for survival in the marine habitat (McCormick et al., 1998; McCormick, 2013). These changes are known as smolting and include morphological, behavioural, biochemical and physiological changes that occur during the spring (McCormick and Saunders, 1987).

Endocrine factors stimulate the physiological changes that occur during smolting. For example, growth hormone, typically associated with increasing body size, also stimulates gill NKA activity and promotes seawater tolerance. Prolactin appears to work in opposition to growth hormone and is typically associated with fresh water acclimation. Cortisol stimulates gill NKA activity and also alters the morphology and development of ionocytes (McCormick, 1995); however, cortisol has a dual role and it is linked to both seawater and fresh water acclimation, and interacts with both growth hormone and prolactin (Shrimpton and McCormick, 1998).

Many of the physiological changes are associated with adjustments in ionregulatory function in specialized cells in the gill known as ionocytes. Ionocytes take up ions from fresh water and secrete ions in seawater (Evans et al., 2005). In both fresh water and seawater the enzyme Na^+/K^+-ATPase (NKA) is important for ionoregulation. Activity of NKA increases with higher salinity of water (McCormick, 1995) and the activity of this enzyme in the gill is often used as an indicator of ability to ionoregulate in

seawater. McCormick et al. (2009) identified that the NKA enzyme differs in Atlantic salmon acclimated to freshwater or seawater; specifically the α1 isoforms. The α1a isoform is found on both the filamental and lamellar ionocytes in freshwater, and α1b is more abundant in seawater and is found principally on large filamental ionocytes (Hiroi and McCormick, 2012). Changes in expression of these two isoforms, along with gill NKA activity, are used as markers to understand changes in osmoregulation during smolting.

The length of time that juvenile salmon are able to survive this transfer to seawater is referred to as the smolt window. If smolts are prevented from reaching seawater within this smolt window, there is a loss of the characteristics associated with marine survival and smolts revert back to a freshwater state (Hoar, 1988; McCormick et al., 1997; 1999). Saltwater exposure after the smolt window can be fatal, as post smolts lack the physiological machinery to regulate ions in a hyperosmotic environment.

Interior populations of salmon must migrate far greater distances than coastal populations to reach the ocean, however the physiological changes associated with smolting appear to occur as fish initiate migration – even in long distance migrating populations of salmon (Chapter 1; Sykes and Shrimpton, 2010). The greater distance for migration and longer time to reach suggests that long-distance migrating populations may require a wider interval of time for the smolt window than short-distance migrating populations. Research on Atlantic salmon (McCormick et al., 1997; 1999) found little difference among populations, however, many populations of Pacific salmon migrate far greater distances and may be a better model to assess population differences in the duration of the smolt window. Work on Pacific and Atlantic salmon has shown that the

smolt window duration is inversely related to temperature – a shorter duration at higher temperatures (Zaugg and McLain, 1976; McCormick et al., 1997). Warmer water temperatures and more variable flows predicted due to climate change, therefore, could potentially put long-distance migrating populations at risk if they do not reach the ocean within the smolt window.

My objective was to determine the effect of temperature on the duration of the smolt window. Sockeye salmon smolts out-migrating from Chilko Lake were captured and held in experimental tanks to determine the effect of temperature on the rate of loss of smolt characteristics. Physiological changes characteristic of smolting were evaluated by quantifying the kinetic activity of gill NKA, and the expression of messenger RNA (mRNA) for the α1a and α1b isoforms for the NKA enzymes in the gill. As well, hormonal activity associated with changing marine environments was examined by quantifying mRNA expression of hormone receptors, receptors for growth hormone, cortisol, and prolactin in the gill.

MATERIALS AND METHODS

Study Area

Chilko Lake is on the eastern side of the Coast Mountain range of British Columbia. Chilko Lake drains north to the Chilko River and then flows into the Chilcotin River before joining the Fraser River in central BC (Fig. 1.1) after 200 km and 821 m elevation drop. Chilko Lake supports a large population of sockeye salmon. In general, sockeye salmon smolts migrate after spending one winter in the Lake, but some rear for an additional year. The outmigration of smolts in 2012 was predominantly year-1 smolts,

as offspring from the high 2010 adult spawning escapement. The Chilko sockeye salmon outmigration typically begins in mid April, and the peak of the run occurs in late April. In 2012, the peak daily smolt outmigration occurred on April 27th, where an estimated 11.7 million smolts moved past the counting fence operated by Fisheries and Oceans Canada (FOC). In addition, a surge of year-1 smolts migrated out a week prior (2.7 million on April 21st).

Experimental Set-up

Fish for the experiment were collected during the first surge of migration on April 21, 2012; year-1 smolts were captured at the FOC enumeration fence during night migration, by dip netting above the fence. University of Northern British Columbia Animal Care and Use Committee approved all experimental procedures, including lethal sampling (UNBC ACUC Protocol Number: 2012-04). Twelve 1-year-old sockeye salmon smolts were sampled immediately after capture to assess size and collect gill tissue prior to experimental manipulation (Table 2.1). An additional 382 1-year-old sockeye salmon smolts were captured and randomly assigned to one of five tanks. Two of the 5 tanks were left at the ambient temperature of the sample site (Chilko River), with flow through water pumped from the Chilko River. The ambient tanks are referred to as A1 and A2. One of the ambient tanks was sampled throughout the experiment (A2) while the other was only sampled at the end of the experiment (A1) to assess the effect of repeatedly sampling, but also to hold fish for potential replacement if there were mortalities in the other treatment tanks; mortalities were negligible and no fish were added to the experimental system after the start of the experiment. The other three tanks were gradually warmed above ambient temperature using electrical aquarium heaters in partial

recirculating systems. The treatment tanks are referred to as T1, T2 and T3, where T3 was the warmest treatment tank. By 48 h after fish were introduced to the tanks, target temperatures for the three experimental treatments tanks were reached. Temperatures of each tank were measured hourly (Water Temp Pro V2, Onset Computer Corporation, Bourne, MA, USA). Average temperatures were 6.7 °C for T1, 10.5 °C for T2, and 15.2 °C for T3 (Table. 2.2 and Fig. 2.1). The ambient tanks, A1 and A2 averaged 4.9 °C and 4.8 °C, respectively. Differences between A1 and A2 were at the peak of daytime temps (Fig. 2.1), linking the 0.1 °C difference to sun exposure of tanks. Maximum temperatures in A1 and A2 were 8.6 °C and 8.4 °C respectively. Maximum temperatures in the treatment tanks T1, T2 and T3 were 12.3 °C, 16.2 °C and 20.2 °C, respectively (Table 2.2 and Fig. 2.1). Accumulated thermal units (ATU) were calculated as the cumulative mean daily temperature. Dissolved oxygen was measured on May 9, and the lowest measured value was 91% of saturation in tank T3 (Oxy-check HI 9147, Hanna Instruments, Woonsocket, Rhode Island, USA). Experiment set-up was outside and fish experienced a natural photoperiod. Water was introduced to each tank at approximately 4 $L \cdot min^{-1}$. For the partial recirculation tanks, river water was added at 0.5 - 1 $L \cdot min^{-1}$. Fish were fed brine shrimp twice daily to satiation (approximately 3% of body $mass \cdot day^{-1}$). Fish (n=12) were sampled every 4 days over the duration of the experiment; total of 7 sample dates (Table 2.1).

Fish and Tissue sampling

For sampling, fish were captured and rapidly transferred to a bucket containing 200 $mg \cdot L^{-1}$ tricaine methane sulphonate (MS-222) buffered with 400 $mg \cdot L^{-1}$ of sodium bicarbonate. The fish were then weighed (to nearest 0.01 g) and measured for

fork length (to nearest mm). Condition factor was calculated as (weight/length^3) X 100000. For determination of gill NKA activity, the first gill arch was removed from the fish, placed on ice, and frozen in liquid N_2 within 5 min. To quantify gill NKA α1 isoforms and hormone receptor mRNA, additional gill tissue (2nd and 3rd gill arches) were collected and submerged in ~600 µL of *RNAlater*. Samples in *RNAlater* were left in the fridge for 24 hours and then transferred to a -20 °C freezer for up to 32 days. Samples were all transferred to a -80 °C freezer at the end of the experiment until analysis.

Analysis of gill Na^+/K^+-ATPase activity

Gill NKA activity was measured according to the microassay protocol of McCormick (1993). Briefly, gill filaments were homogenized in SEI buffer (150 mM sucrose, 10 mM Na2EDTA, 50 mM imidazole, pH 7.3) containing 0.1 % sodium deoxycholate (SEID). Centrifugation (5000 g for 30 seconds) produced the supernatant which was used to determine the enzyme activity by relating ATP hydrolysis to the oxidation of nicotinamide adeninedinucleotide (NADH), measured at 340 nm for 10 minutes at 25 °C, replicated twice in the presence and absence of 0.5 mmol · L^{-1} ouabain on a plate reader (VersaMax, Molecular Devices, Sunnyvale, California, USA). Protein content was then measured using a bicinchoninic acid (BCA) protein assay (Pierce, Rockford, Illinois, USA). Specific activities were expressed as µmol ADP · mg^{-1} of protein · h^{-1}.

Purification of total RNA from gill tissue and cDNA synthesis

Total RNA was extracted from gill tissue (approximately 5-35 mg) using *RNeasy Mini Kit* (Qiagen, Mississauga, ON). Samples were homogenized in a Geno/Grinder 2000 (BT&C, Inc., Burlington, ON). Isolated RNA was dissolved in 40 µL RNase-free

deionized water. The concentration and purity of each RNA sample was determined by measuring optical absorbance at 260 and 280 nM on a Nanodrop spectrophotometer (Nanodrop Technologies, Wilmington, Delaware, USA). To assess RNA integrity, a subset of samples were run on the Bio-Rad Experion Automated Electrophoresis Station (Bio-Rad Laboratories, California, USA). Purified RNA was converted to cDNA using Qiagen's *QuantiTect Reverse Transcription Kit* (Protocol: Reverse Transcription with Elimination of Genomic DNA for Quantitative, Real-Time PCR).

Primers and Real-Time PCR

Messenger RNA was measured for genes involved in ionoregulation using quantitative Real-Time Polymerase Chain Reaction (qRT-PCR). The isoforms of NKA give insight into fresh water to seawater transition, as the α1a isoform is associated with freshwater, and the α1b isoform with seawater (Richards et al., 2003). Primers derived from *O. mykiss* were used to target α1a from Madsen et al. (2008) and α1b from Richards et al. (2003). Endocrine signals throughout the experiment were assessed by measuring mRNA for cortisol, prolactin and growth hormone receptors. Cortisol was evaluated by measuring the Glucocorticoid Receptor1 receptor (GR1); primers designed for *O. mykiss* by Sathiyaa and Vijayan (2003) were used. Prolactin receptor (PrlR) primers were designed for *O. mykiss* by Kiilerich et al. (2007) and growth hormone receptor 1 (GHR1) by Very et al. (2005). Relative gene expression was standardized to the reference gene, β-actin using primers from Sathiyaa and Vijayan (2003). Although primers were designed for *O. mykiss*, all primers were validated for *O. nerka* and verified that they amplified the gene of interest (Flores et al., 2012).

All qRT-PCR reactions contained 1 μL of cDNA template, 4 pmoles of each isoform specific primer and Universal SYBR green master mix (Applied Biosystems Inc., Carlsbad, California, USA). All qRT-PCR reactions were 2 minutes at 50 °C, 10 minutes at 95 °C, followed by 40 cycles of 95 °C for 15 seconds and 60 °C for 1 minute using an Applied Biosystems Inc. 7300 Real-Time PCR System (Carlsbad, California, USA). Melt curve analysis was performed after each reaction to confirm the presence of only a single product of the reaction. RNA controls were also run on the RT-PCR system. To test for genomic DNA contamination, samples of RNA that were not reverse transcribed were analyzed. Genomic DNA contamination was present in 15% of samples tested, but it was never more than 1:37000 starting copies for NKA α1a-isoform, not detected for NKA α1b-isoform, 1:58000 starting copies for β-actin, 1:1900 for GR1, 1:1100 copies for GHR1, and not detected for PrlR. Genomic DNA, therefore, was found to be negligible.

Randomly selected samples were serially diluted to develop a standard curve relating threshold cycle to cDNA amount for each primer set. The slopes were linear and similar for all genes, suggesting that the amplification efficiency in the qRT-PCR reactions did not differ between genes (Flores et al., 2012). Therefore, the relative expression of the target genes could be normalized to a reference gene by utilizing the $\Delta\Delta C_T$ method (Livak and Schmittgen, 2001). Gene expression levels measured in qRT-PCR assays were normalized to the mRNA level of the reference gene, β-actin. To verify normalization of qRT-PCR results with β-actin, mRNA from a second reference gene, elongation factor 1a, was also tested on a subset of samples; primers from Richards et al., 2003. Results did not differ significantly between the two reference genes. All samples were run in duplicate and the standard deviation between duplicate C_T values never

exceeded 0.5. The mRNA copies were expressed relative to the gill samples collected from smolts collected at the fence on the initial day of the experiment. Samples were run from ambient tanks; A1 and A2, and only the warmest treatment tanks; T2 and T3.

Statistical Analysis

Analysis of variance (ANOVA) tests were performed to compare mean differences. Multiple comparisons of means were also performed (Tukey's Contrasts). Linear model regression was performed to analyze trends in relation to the date of collection. For graphing purposes, a Loess (Local Regression) smooth line was locally fitted to NKA activity and gene expression by accumulated thermal units (ATU). Statistical significance was taken at a level of $p < 0.05$. All statistical analysis was performed in R software (ver. 3.0.2). All values are expressed as means ± 1 standard error (se).

Table 2.1. Date and number of sockeye salmon were sampled from each group

Sample Date	Group					
	A1	A2	T1	T2	T3	Fence
Apr 21						12
Apr 25		13	11	12	12	
Apr 29		12	12	13	12	
May 03		12	12	12	13	
May 07		12	12	12	12	
May 11		12	12	13	12	
May 15		12	12	12	12	
May 18	12	12	14	18	23	

Table 2.2. Minimum, mean and maximum temperature (°C) of treatment tanks, 48 h after fish were introduced to tanks.

	Tank				
	A1	A2	T1	T2	T3
Min	2.4	2.4	3.1	6.1	9.0
Mean	4.9	4.8	6.7	10.5	15.2
Max	8.6	8.4	12.3	16.2	20.2

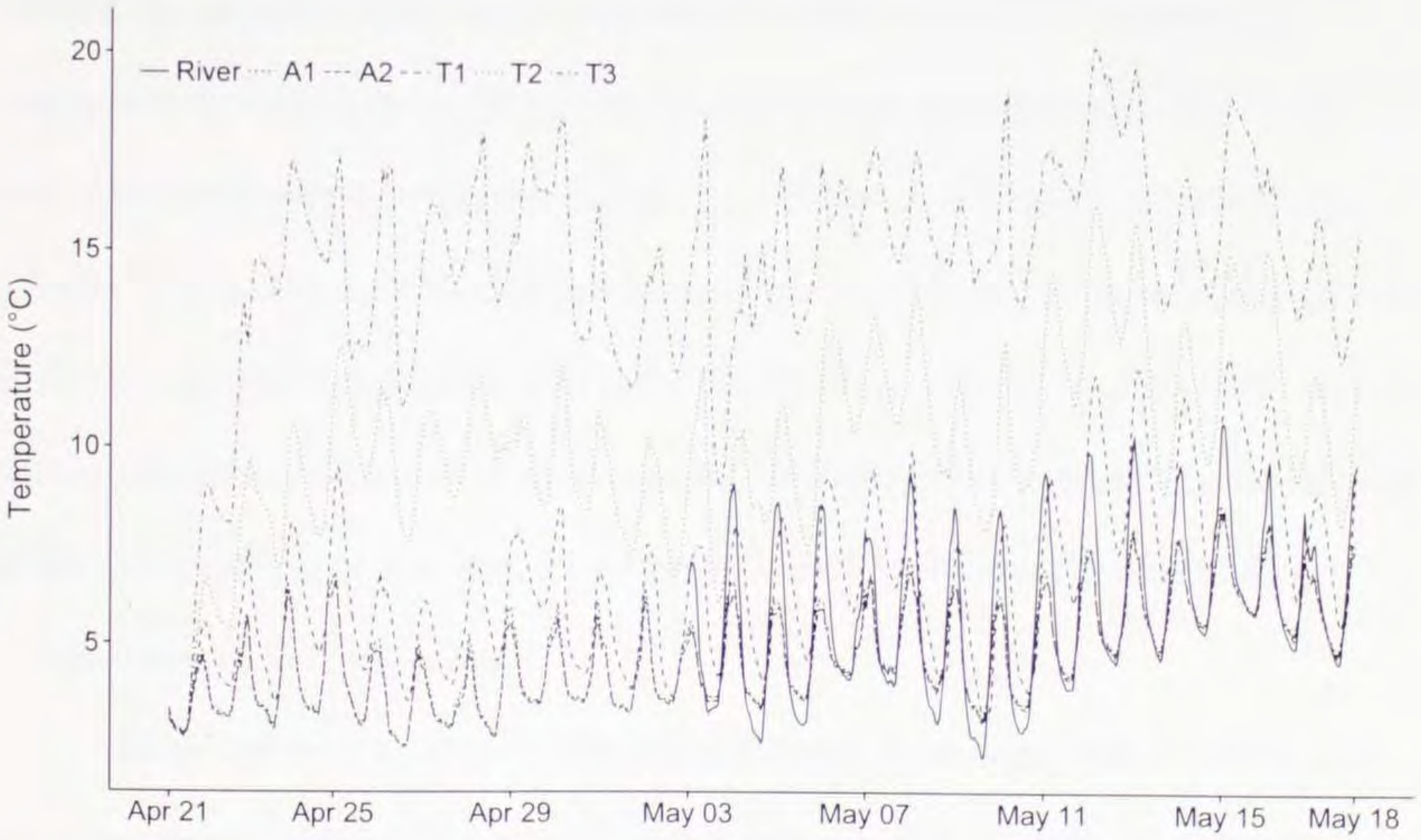

Figure 2.1: Temperatures of Chilko River, Ambient tanks (A1 and A2) and treatment tanks (T1, T2, T3). Hourly temperature in °C.

RESULTS

Length, weight and condition factor

Length did not differ significantly among treatment tanks (F_5=0.321, p=0.9) or by date (F_7=1.24, p=0.28) (Fig. 2.2A). Fish weight also did not differ among tanks (F_5=1.26, p=0.28) or by date (F_7=1.47, p=0.18) (Fig. 2.2B). Fish weights were lower in the warmest experimental tank (T3) on the final day of the experiment (May 18); however, the weights did not differ significantly from the fish weights on previous dates of the experiment. Condition factor (K) of fish changed during the experiment. Changes in T2 and T3 occurred over the sampling period (T2: F_6=3.05, p=9.54x10^{-3}; T3: F_6=24.25, p=2x10^{-16}) (Fig. 2.2C). On the last day of the experiment (May 18), there were significant differences in condition factor across tanks (F_4=20.91, p=1.45x10^{-11}). Fish from T2, were only significantly different from the fish in A2 (t=-2.95, p=0.03). As well on the last day of the experiment, K of fish from T3 was significantly lower than all other tanks (all comparisons p<0.001) (Fig. 2.2C).

There was not a significant difference in length or weight relative to ATU over the experiment. Condition factor however did decrease with ATU (F_{28}=8.38, p=2x10^{-16}; data not shown).

NKA activity, α1a and α1b isoforms

Gill NKA activity showed a moderate increase with time after capture and transfer to the experimental tanks (Fig. 2.3A). There was no difference across time in the ambient tank (A2; F_6=0.77, p=0.6) or tank T1 (F_6=0.78, p=0.58). Gill NKA activity in fish from T2 stayed stable throughout the experiment with the exception of a significant difference between peak NKA activity (May 07) and the last day of the experiment (t=-

3.35, p=0.02). Conversely, a pronounced decrease in gill NKA activity over time was observed in T3 (F_6=47.69, p=2x10^{-16}). Divergence from the other tanks occurred on May 03. A significant negative regression was observed (R^2=0.74; $F_{6,86}$=47.69, p=2.2x10^{-16}) from the onset of the experiment. There were also significant differences observed in the α1a isoform over the sampling period (F_7=6.67, p=6.46x10^{-7}) (Fig. 2.3B). The most substantial differences were between Apr 25 and the initial sampling (Apr 21), and on day 13 and day 17 (May 03 and May 07), where highest values were observed on Apr 25. Significant differences in the tank means were not observed for the α1a isoform (F_4=1.94, p=0.11). The α1b isoform also differed over the sampling period (F_7=5.63, p=8.08x10^{-6}) (Fig. 2.3C). A decrease from the initial sampling day (Apr 21) occurred right after capture and remained low for the rest of the experiment. During the temperature treatments, there was a significant difference between Apr 25 and May 15. Significant differences in the tank means were observed for the α1b isoform (F_4=15.71, p=6.99x10^{-11}). A significant decrease was observed between the fence and the other tanks. As well, the treatment tanks (T2 and T3) were significantly lower than the ambient tank (A2).

There was also a relationship between gill NKA activity and ATU (F_{28}=21.26, p=2x10^{-16}) (Fig. 2.4A). There was a slight initial increase in gill NKA activity and the fitted LOESS line suggested a peak at 177 ATU and then a subsequent decline with the lowest gill NKA activity observed at the highest ATU of 393 (Appendix 2.1). The NKA α1a isoform also changed with ATU (F_{22}=21.26, p=4.67x10^{-12}) (Fig. 2.4B). An initial increase occurred from the start of the experiment and peaked 30-44 ATU (Appendix 2.2). Following peak abundance of NKA α1a isoform, levels decreased with a low at 105 ATU, and then a moderate increase with higher ATU. The NKA α1b isoform also changed with

ATU (F_{22}=7.39, p=9.97x10^{-15}) (Fig. 2.4C). The decrease in the NKA α1b isoform was not directly related to ATU as it occurred initially with the treatment tanks (T2 and T3). A decrease from the onset also occurred in ambient tank (A2), however this was delayed. Fluctuations occurred around the decrease, particularly in A2 and T3 (Appendix 2.3).

Growth Hormone, Cortisol and Prolactin receptor mRNA

Growth hormone receptor 1 (GHR1) mRNA levels also differed over the sampling period (F_7=7.89, p=3.6x10^{-8}) (Fig. 2.5A) with a significant decrease after capture and then low GHR1 mRNA for the remainder of the experiment. Significant differences in the tank means over the course of the experiment were observed for GHR1 (F_4=15.89, p=5.9x10^{-11}). These differences were between the initial sampling at the fence and the other tanks. As well, over the course of the experiment T3 was significantly lower than the ambient tank (A2). Glucocorticoid receptor 1 (GR1) mRNA levels did not differ over the sampling period (F_7=1.24, p=0.29) (Fig. 2.5B). Similarly, there were no significant differences in the tank means over the course of the experiment for GR1 (F_4=1.04, p=0.39). Prolactin hormone receptor (PrlR) mRNA levels did not differ over the sampling period (F_7=1.9, p=0.07) (Fig. 2.5C). Although, significant differences in the tank means were observed for PrlR (F_4=3.3, p=0.01), the only difference observed was between the ambient tank (A1; sampled once at the end of the experiment) and the warmest treatment tank (T3).

Growth hormone receptor 1 (GHR1) mRNA levels changed over the course of the experiment in relation to ATU (F_{22}=4.11, p=1.3x10^{-07}) (Fig. 2.6A). A decrease occurred after the onset, and GHR1 remained low for the duration of the experiment (Appendix 2.4). GR1, however, did not change relative to increases in ATU (F_{22}=1.42, p=0.12) (Fig.

2.6B). Prolactin receptor changed relative to ATU (F_{22}=2.77, p=1.62x10^{-4}). Significant differences were observed from the high points at 15, 237 and 272 ATU with the low observed at 44 ATU (Appendix 2.5), however, little pattern was evident for PrlR and ATU.

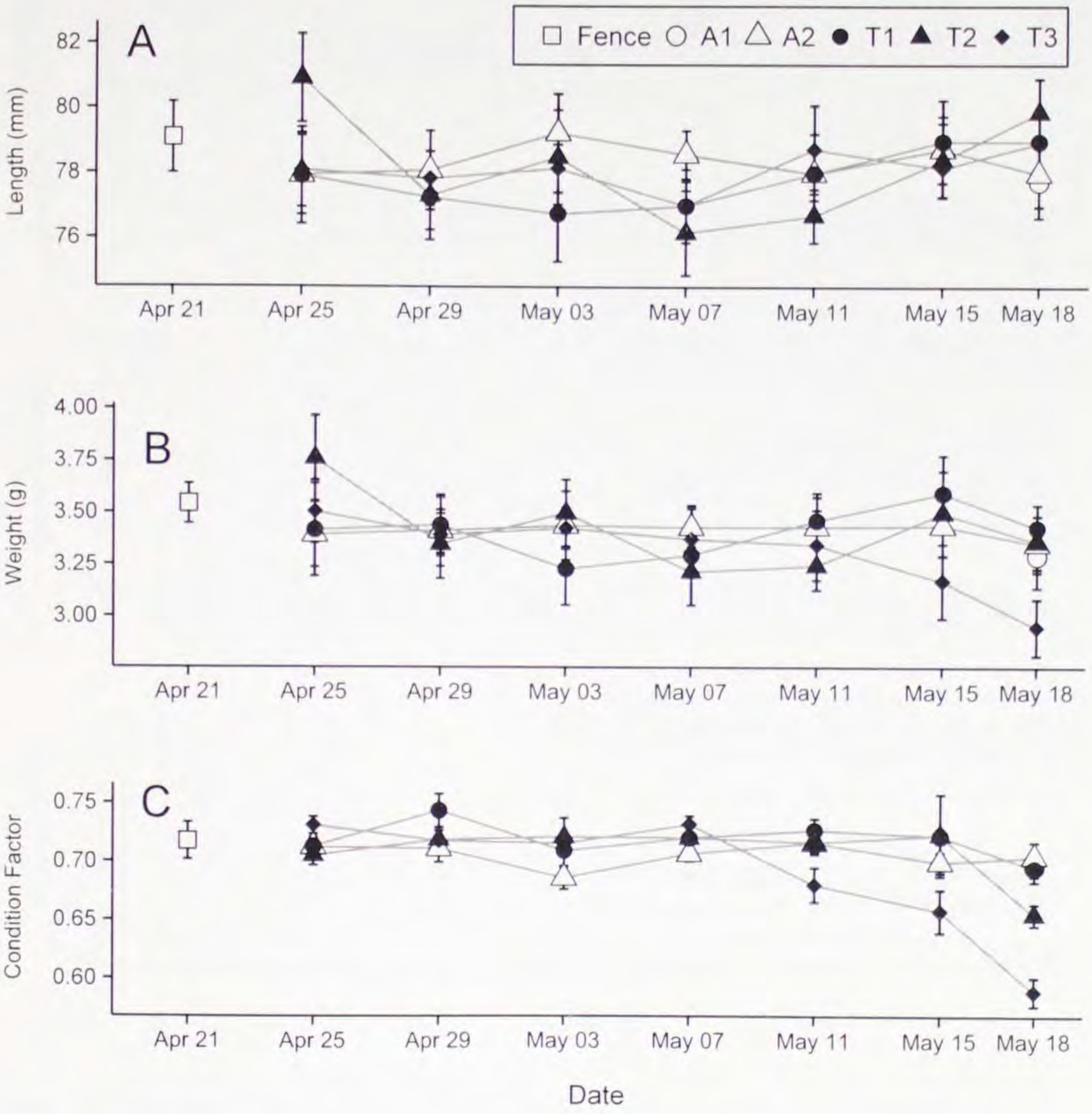

Figure 2.2: (A) Length, (B) weight and (C) condition factor by date of sampling (2012). Initial sampling at the fence at the onset of experiment (open squares), ambient tank 1 (open circles), ambient tank 2 (open triangles), treatment tank 1 (solid circles), treatment tank 2 (solid triangles) and treatment tank 3 (solid diamonds). Means ± SE.

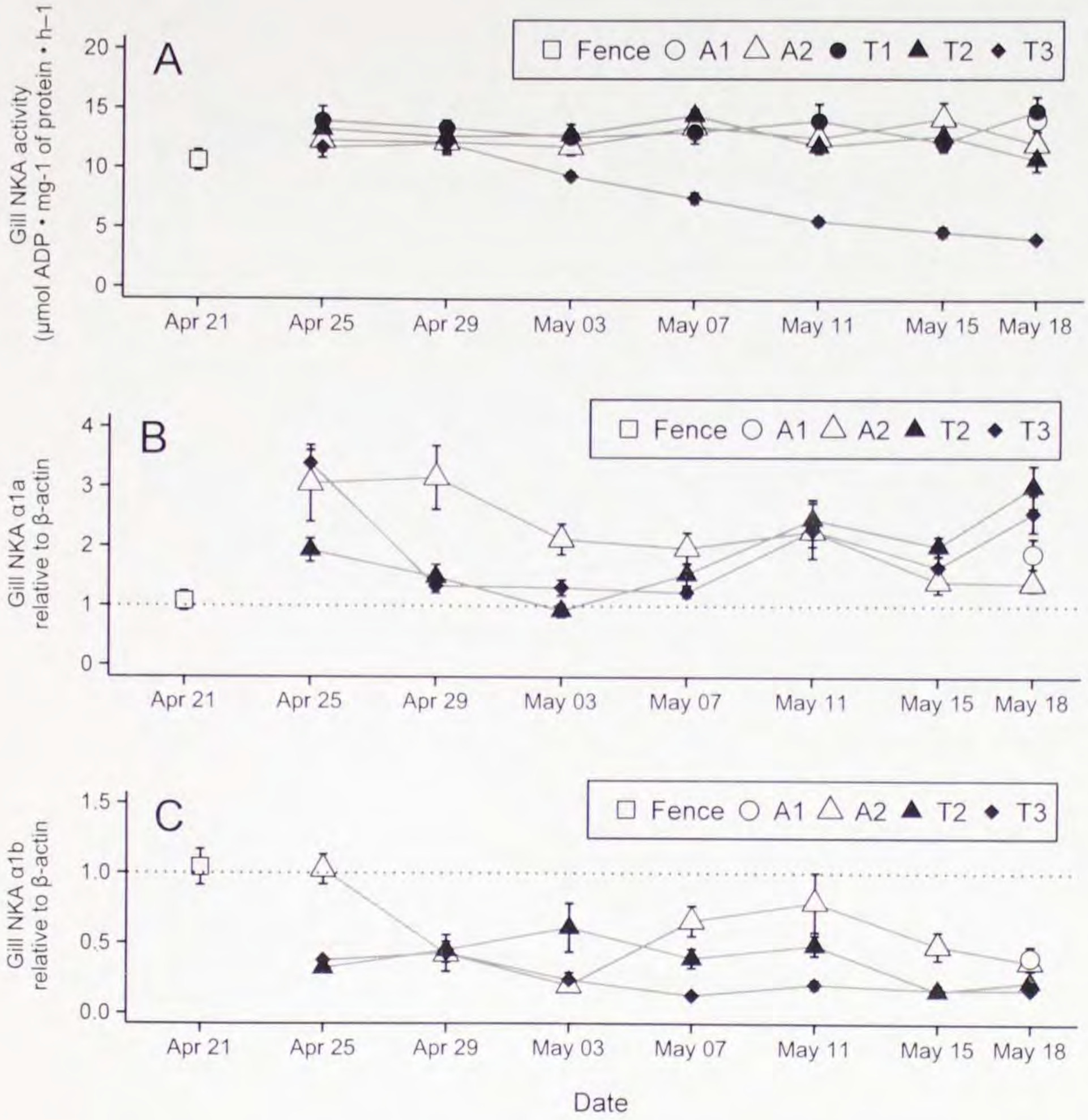

Figure 2.3: Changes in (A) Gill Na^+/K^+-ATPase (NKA) activity (µmol ADP • mg^{-1} of protein • h^{-1}), (B) gill NKA α1a expression (relative to β –actin) and (C) gill α1b expression (relative to β –actin) by date of sampling (2012). Initial sampling at the fence at the onset of experiment (open squares), ambient tank 1 (open circles), ambient tank 2 (open triangles), treatment tank 1 (NKA activity only; solid circles), treatment tank 2 (solid triangles) and treatment tank 3 (solid diamonds). Means ± SE.

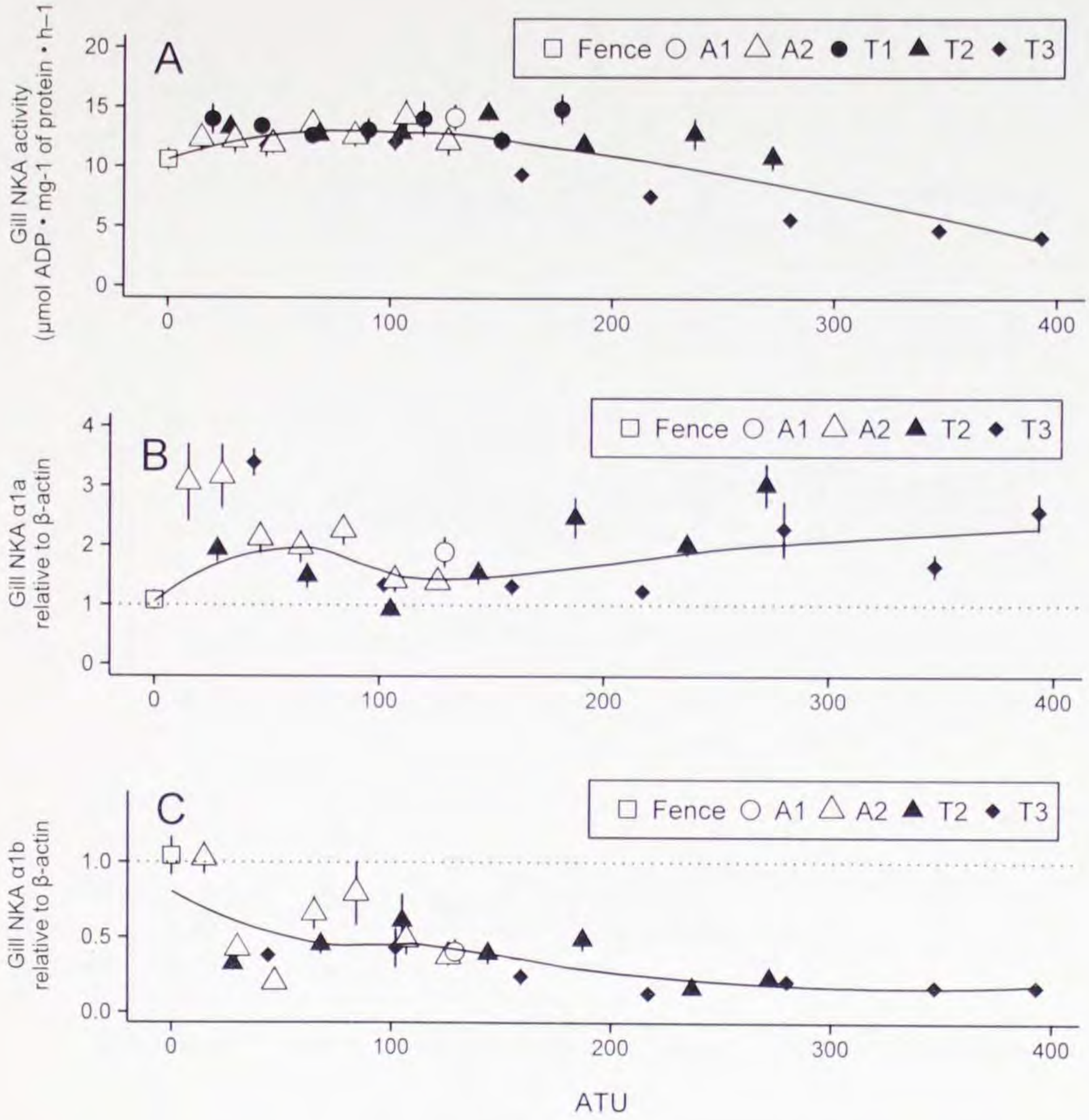

Figure 2.4: Changes in (A) Gill Na^+/K^+-ATPase (NKA) activity (μmol ADP • mg^{-1} of protein • h^{-1}), (B) gill NKA α1a expression (relative to β–actin) and (C) gill α1b expression (relative to β –actin) by accumulated thermal units (ATU) of experiment. Initial sampling at the fence at the onset of experiment (open squares), ambient tank 1 (open circles), ambient tank 2 (open triangles), treatment tank 1 (NKA activity only; solid circles), treatment tank 2 (solid triangles) and treatment tank 3 (solid diamonds). Means ± SE. Fitted LOESS line to data (solid line).

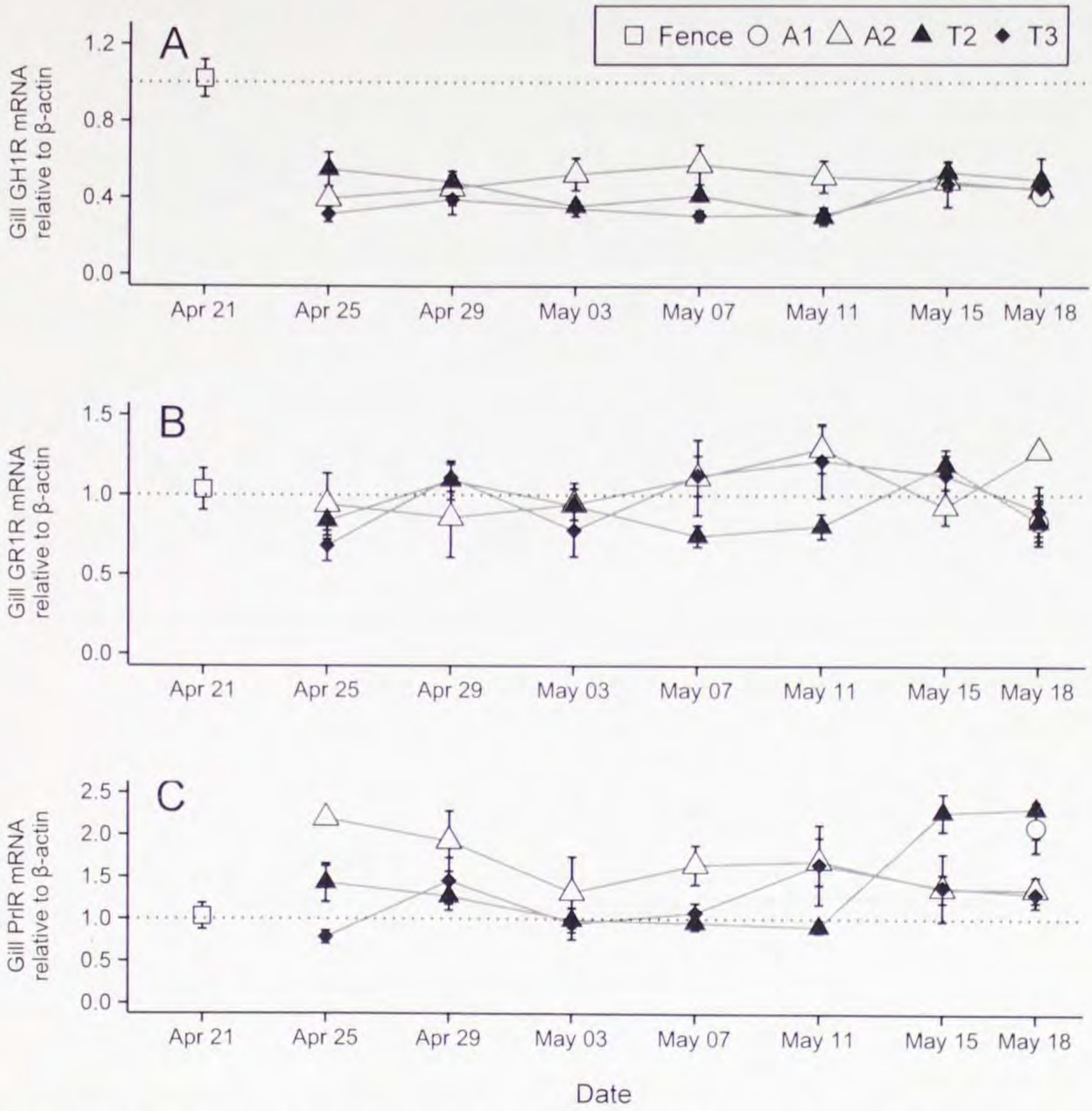

Figure 2.5: Changes in (A) gill growth hormone receptor 1 (GHR1) mRNA expression (relative to β –actin), (B) gill glucocorticoid receptor 1 (GR1) expression (relative to β –actin) and (C) gill prolactin receptor (PrlR) expression (relative to β –actin) by date of sampling (2012). Initial sampling at the fence at the onset of experiment (open square), ambient tank 1 (open circles), ambient tank 2 (open triangles), treatment tank 2 (solid triangles) and treatment tank 3 (solid diamonds). Means ± SE.

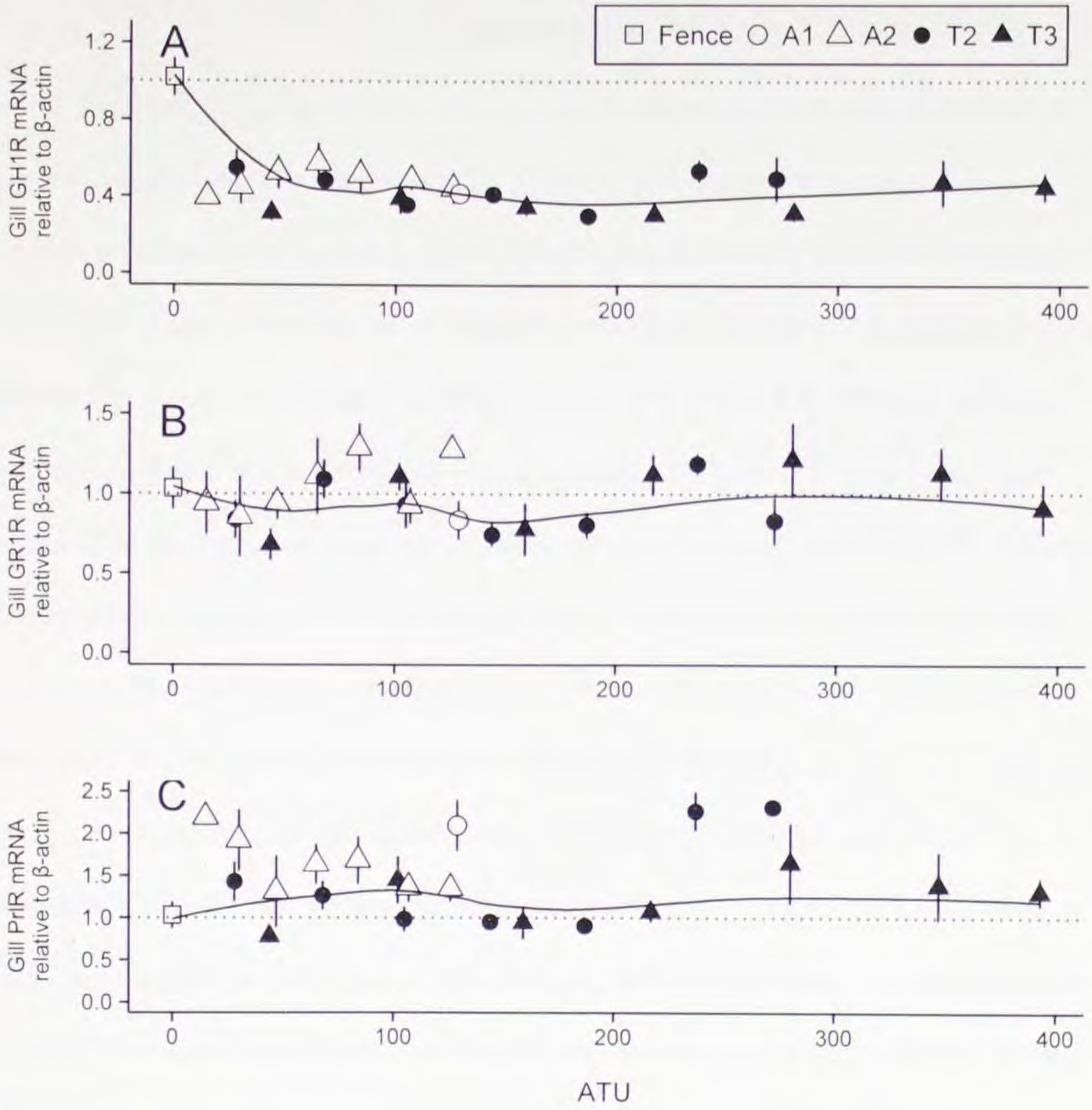

Figure 2.6: Changes in (A) gill growth hormone receptor 1 (GHR1) mRNA expression (relative to β –actin), (B) gill glucocorticoid receptor 1 (GR1) expression (relative to β –actin) and (C) gill prolactin receptor (PrlR) expression (relative to β –actin) by accumulated thermal units (ATU) of experiment. Initial sampling at the fence at the onset of experiment (open squares), ambient tank 1 (open circles), ambient tank 2 (open triangles), treatment tank 2 (solid triangles) and treatment tank 3 (solid diamonds). Means ± SE. Fitted LOESS line to data (solid line).

DISCUSSION

My study has demonstrated that juvenile sockeye salmon migrating from their natal lake appear to be competent smolts. Further, the behavioural and physiological changes associated with smolting appear to occur synchronously in smolts emigrating from Chilko Lake. The initiation of migration and active downstream movement suggests that the fish were behaviourally smolting. Fish caught at the Chilko River downstream enumeration fence also exhibited gill NKA activities that were at or near peak values measured in the study and suggests the fish were physiologically smolting. This finding is in agreement with data on juvenile sockeye salmon presented in Chapter 1 where fish were intercepted at the onset of migration at other locations within the Fraser River catchment. In that study juvenile sockeye salmon leaving their natal lakes also had NKA levels characteristic of smolts. Interestingly, the highest gill NKA levels were consistently observed for sockeye salmon populations from interior lakes with long migration distances to the ocean; coastal lake populations were more variable. Juvenile Atlantic salmon have also been shown to exhibit behavioural and physiological changes characteristic of smolts at the onset of migration (McCormick et al., 1998). Additionally work on Chinook salmon in a laboratory study on an Upper Fraser River population indicated synchrony between physiological and behavioural changes that occur during the parr-smolt transformation (Sykes and Shrimpton, 2010).

What limits the duration of the smolt window?

The length of time that juvenile salmon are able to survive transfer to seawater during the spring is transient (McCormick et al., 1997; Shrimpton et al., 2000). The findings in the study show that the length of time can be abbreviated in warm water

temperatures. Interior populations must migrate far greater distances than coastal populations, but as I have shown in Chapter 1 and also with the present study the physiological changes associated with smolting occur as fish initiate migration; for the Chilko Lake population this is a distance of 684 km from the ocean. It is expected, therefore, that long distance migrating populations should have a longer interval of time for the smolt window than short distance migrating populations. Although, the duration of the smolt window has not been examined in coastal sockeye salmon populations, the smolt window for the Chilko Lake population appears to exceed 4 weeks duration in the present experiment. The duration of the smolt window, however, has been shown to be inversely related to temperature – a shorter duration at warmer temperatures (Adams et al., 1973; Zaugg and McLain, 1976; Zaugg 1981; Duston et al., 1991; McCormick et al., 1997, Handeland, 2014). The mechanism for the loss of smolt characteristics appears to be linked to the thermal experience or ATU (McCormick et al., 1997). The effects of temperature on smolt related characteristics were also evident in the present experiment. Warm temperature abbreviated the smolt window as indicated by an earlier decrease in gill NKA activity. Similarly, holding fish in cooler water during the parr-smolt transformation can also lengthen the smolt window (McCormick et al., 1997; 1999).

Data from my study, however, does not appear to support the model that ATU defines the duration of the smolt window. Only the warmest temperature group showed a significant decline in gill NKA. The decline in gill NKA was observed after approximately 200 ATU. McCormick et al. (1997) showed the decline at 300 ATU. Additionally, in my study the mid temperature group maintained high levels of gill NKA for almost 300 ATU; the value of ATU for which McCormick et al. (1997) observed a

50% decline in gill NKA activity for Atlantic salmon (*Salmo salar*) smolts (McCormick et al., 1997). My findings, therefore, suggest that an upper threshold temperature may have been reached that resulted in the loss of smolt characteristics. A threshold temperature for initiation of smolt migration has previously been suggested (e.g., Solomon, 1978), but this paradigm has been replaced with models that suggest thermal experience is more important for stimulating downstream movement (Zydlewski et al., 2005; Sykes et al., 2009).

Whether the mechanism controlling the loss of smolt characteristics is common for all anadromous species of salmon or even consistent among populations of the same species is not known. Life history variation among anadromous salmonids suggests that differences may exist – the effect of temperature may differ due to the range of potential thermal regimes juvenile salmon experience. Atlantic salmon parr use habitats ranging from riffles to pools and lakes within the stream system and even to estuaries (McCormick et al., 1998). Chinook and coho salmon parr move downstream and into larger river systems prior to the parr-smolt transformation and migration to the ocean (Shrimpton et al., 2014). These patterns of migration differ from sockeye salmon, where parr reside in a lake and do not leave this environment until they smolt, and begin actively migrating to the ocean. As a result, the thermal experience in the spring for sockeye salmon smolts may differ from fluvial species of anadromous salmon. A recent study found that rearing environment of pre-smolts affects migration timing. The migration period of Arctic char from northern Norway were shown to have a smaller range and less annual variation than Atlantic salmon and brown trout *Salmo trutta* (Jensen et al., 2012). The authors speculated that the difference was related to the lake-

dwelling habitat preference of Arctic char and photoperiod was most important for explaining Arctic char movement. Whereas, the *Salmo* species that reside in fresh water may have adapted to more variability in water temperatures from residing in a variety of habitats, the accumulated thermal experience may be driving physiological changes. In the case of juvenile sockeye salmon, where spring temperatures are less variable in lakes, the effect of warm temperatures may have a more acute effect on loss of smolting. In this experiment, an abbreviated smolt window was observed for fish in the warmest temperature group. Gill NKA activity declined approximately 7 days after the warmest temperature tank achieved an average temperature of 14.6 °C (April 23). Prior to the decline (Fig. 2.3A), the maximum temperature fish had experienced was 18.3 °C and occurred 3 days previously on Apr 30 (Fig. 2.2).

Is ionoregulatory physiology static during the smolt window?

Gill NKA for most of the treatment groups showed little change throughout the experiment, however, the α1a and α1b isoforms of NKA in the gill showed considerable changes with time and ATU. The α1a isoform increased from the onset of the experiment and the α1b decreased. Furthermore, the changes occurred right at the onset of the experiment, within the first 4 days. Interestingly, the α1a isoform, which is the isoform associated with freshwater ionoregulation (McCormick et al., 2009; Flores and Shrimpton, 2012) immediately increased and then fluctuated above the level measured at the onset of the experiment. Similarly, the α1b isoform, which is associated with seawater ionoregulation (McCormick et al., 2009; Flores and Shrimpton, 2012) decreased and stayed below the level measured at the onset. The decrease, however, was slightly delayed in the ambient water group. The cessation of migration may have driven the

changes in the α1b isoform. Disruption from the behavioural aspect of migration may have been the cue to revert back to the optimal freshwater state. The importance of flow on successful migration of smolts to the ocean has been well established (Raymond, 1979; Conner et al., 2003), but not on the initiation of smolting (Sykes and Shrimpton, 2010). It is unlikely, therefore, that sustained flow and a fluvial environment is essential for maintaining seawater tolerance in smolts – particularly given the numerous studies that have shown successful smolt development in laboratory experiments.

In general, the α1a isoform increased but was variable, whereas the α1b isoform decreased and stayed low. During the smolt window, fish are able to survive in both fresh water and seawater simultaneously. McCormick et al. (2013) attribute this ability to the different α1 isoforms of NKA in the gill. They found that the major change during smolting was the upregulation of the α1b isoform – not a decline in the α1a isoform. Consequently, smolting is adaptive for seawater entry and does not appear to be maladaptive for freshwater residence (McCormick, 2013). McCormick et al. (2013) showed that the abundance of the α1b isoform decreased and went back to pre-smolt levels if fish were maintained in freshwater, however, abundance of the α1b isoform increased following transfer to seawater. Clearly the α1b isoform is important for seawater survival and the smolt window represents a period of time when physiological function is dynamic.

Upregulation of seawater tolerance while fish are still in fresh water indicates a mechanism that prepares fish to survive in higher salinity triggered by changes in environment. Responsiveness to environmental signals, however, differs with stage of development. Seasonal changes in biochemical and endocrine factors were observed in

yearling Atlantic salmon, but the magnitude of change was dependent on size and only the larger size class became competent smolts (Shrimpton et al., 2000). Recently, parr were shown to have a moderate increase in the α1b isoform following seawater transfer, however, they were not able to survive seawater transfer – although time to mortality increased during the spring (McCormick, Regish and Christensen, unpublished data). Consequently, the seasonal changes in physiology are dynamic and the developmental basis to smolting is related to size (McCormick et al., 2007). Variation in the number of age 1 verses age 2 sockeye salmon smolts that migrate from Chilko Lake annually suggests size may also influence development in sockeye salmon.

The upregulation of the seawater excretory ionocytes and enzymes during freshwater residence minimizes the perturbation to osmoregulation when fish migrate into the marine environment. If fish are not competent smolts when they enter seawater, physiological function will be impaired. For example, swimming performance suffers when fish are not capable of ion regulating in seawater. Coho parr held in seawater have high plasma ions and also exhibit poor oxygen delivery, and low muscle moisture, which decreases swimming performance (Brauner et al., 1992). In competent Atlantic salmon smolts, however, gill NKA activity was high and muscle moisture was maintained within narrow range during freshwater to seawater transition (Stefansson et al., 2003). Physiological changes continue to occur during the smolt window, upregulation of seawater ionoregulation will limit declines in performance.

Do hormonal signals reveal factors regulating loss of smolting?

Endocrine signals were affected by the temperature treatments during the course of the experiment. Similar to the α1 isoforms, the pattern of change in mRNA for hormone receptors in the gill varied more closely with ATU than time. Copies of mRNA signalling for GHR1 dropped immediately after the initiation of the experiment. The drop in an endocrine signal important for seawater adaptation, however, will not necessarily cause the smolts to revert to parr. Numerous studies have shown that juvenile salmon treated with GH show increased gill NKA activity and a proliferation of ionocytes (McCormick, 1995; McCormick, 2001). The decrease in GHR1 mRNA immediately after transfer to the experimental tanks suggests that the signal for seawater acclimation had decreased, but the change in signal was not likely to drive parr-reversion immediately. For example, the delay between growth hormone treatment and elevated gill NKA activity has been shown to be at least 7 days (Shrimpton and McCormick, 1998) or more (Shrimpton et al., 1995). Temperature and time did not appear to have an effect on cortisol as indicated by little to no change in GR1 mRNA. Copies of PrlR mRNA showed a moderate increase at the onset of the experiment. Increases in PrlR would account for the increases in the α1a isoform and a strong relationship between mRNA for gill NKA α1a and gill PrlR has been shown for adult sockeye salmon migrating into fresh water (Flores et al., 2012). It is also possible that consistent levels of cortisol in the system may have muted the effects of prolactin (McCormick, 2013). It appears, therefore, that the decrease in gill mRNA for GHR1 suggests lower plasma GH levels that result in a decrease in gill mRNA for the NKA α1b isoform. The decline in endocrine signal, however, does not translate into an immediate decline in gill NKA activity.

Significance

Results of my study suggest that fish are passing through the fence as smolts, but physiological function in fish during the smolt window is not static – and yet smolts must reach seawater within the smolt window. Tagging studies of Chilko smolts show that smolts take approximately one week to migrate from the fence to the ocean (Rechisky et al., 2013). In this study, I demonstrated that the time after fish became competent smolts was limited by high temperature. Changes in mRNA levels of the α1 isoforms and gill PrlR and GHR1 occurred soon after sockeye salmon smolts migrated out of the lake. Warmer temperatures, however, abbreviated the time between the decrease in endocrine signal and decline in gill NKA activity. The effect of holding fish at different temperatures did not appear to be a function of the average thermal experience, but rather the high temperature appears to exceed a threshold for NKA activity and it dropped in the highest temperature group. A shortened smolt window, driven by warming temperatures could mean that fish will enter seawater after reverting to a freshwater state, and losing the machinery to survive in seawater. Mean temperature increases have been observed in the Fraser River in recent years (Morrison et al., 2002), but when those mean increases are applied to current out-migration temperatures, they do not appear to exceed the threshold temperature determined in the present study. Consequently, Chilko Lake sockeye salmon may be able to reach the ocean under a thermal regime that does not result in a shortened smolt window.

EPILOGUE

Physiological changes that occur in the spring are preparatory for salmon smolts to successfully enter seawater. The differences in smolt physiology, relative to migration distance, timing, or during migration were examined in Chapter 1. Parr, or pre-migratory fish had low levels of gill Na^+/K^+-ATPase (NKA) activity. High gill NKA activities were observed at the start of migration for some populations; however, smolts leaving the lake did not consistently have higher gill NKA activity than non-migratory juvenile sockeye salmon sampled in their natal lakes. There was no relationship between gill NKA at the start of migration and distance to the ocean, but gill NKA activity varied with time for smolts leaving their natal lake. Gill NKA activity changes during downstream migration were also highly variable, but consistently smolts in the ocean had the highest gill NKA activities. Internal and external factors may influence this variation, but the dynamic nature of smolting was not based on the region of origin, timing during migration, or on the year of migration.

Environmental factors such as temperature; influence the duration of the smolt window. In chapter 2, I tested the effect of warm temperature on the length of the smolt window for migrating sockeye salmon smolts on the Chilko River. Aside from the warmest treatment, gill NKA activity remained high throughout the study. As well, the decrease appeared to be a result of exceeding a threshold temperature rather than in response to accumulated thermal units (ATU).

At the onset of migration, shown in both chapter 1 and chapter 2, fish left their natal lake with gill NKA activity levels, characteristic of competent smolts. In chapter 1, temporal variation in size and developmental stage of smolts at the start of migration were measured on four sample dates; April 21 (n=12), April 30 (n=13), May 8 (n=11),

and May 18 (n=11). Smolts varied in size at the start of migration; early and late migrants were larger than fish leaving the lake at the peak (Table E.1). Additionally, condition factor was significantly higher for fish sampled on the last date suggesting that the later migrating fish had higher energy reserves and that energetic status was different among smolts initiating migration. Although sizes differed, there was no trend between sample date and gill NKA activity suggesting no relationship between smolt competence and migration timing. To further understand smolt physiology at the onset of migration, mRNA for the NKA α1 isoforms were quantified, along with mRNA for three hormone receptors in the gill; the receptors for growth hormone (GHR1), cortisol (Glucocorticoid receptor 1 (GR1)) and prolactin (PrlR). The NKA α1 isoforms (α1a and α1b) further support little effect of migration timing on smolt characteristics; there was no difference in mRNA expression of gill NKA α1a or NKA α1b isoform with sampling date. Similarly, both GR1 and PrlR mRNA did not differ over the start of migration. Growth hormone receptor 1 mRNA levels, however, changed with sample date for fish leaving Chilko Lake. The smolts captured at the fence on the last sampling date exhibited levels of GHR1 expression significantly lower than the fish captured on the first two sampling dates. At the start of migration, therefore, smolt development appeared to be consistent, except the mRNA data suggests that GH may be declining for late migrating smolts. Lower mRNA levels for GHR1 suggests lower levels of growth hormone and these fish may be already further along in the smolt window when they initiated migration.

Table E.1: Changes in smolts over the start of migration captured at the Chilko enumeration fence in 2012. Length (mm), weight (g), condition factor, Gill Na^+/K^+-ATPase (NKA) activity (μmol ADP • mg^{-1} of protein • h^{-1}), gill NKA α1a and α1b expression (relative to β–actin), gill growth hormone 1 receptor (GH1R) mRNA expression (relative to β–actin), gill glucocorticoid receptor 1 (GR1) expression (relative to β–actin) and gill prolactin receptor (PrlR) expression (relative to β–actin). Means ±SE. Significant p-values are bolded. Values with a common letter do not differ significantly.

	F_3 p	Apr 21	Apr 30	May 8	May 16
length	5.92	79.1 ± 1.1^{a}	76.5 ± 1.2^{ba}	72.2 ± 2.3^{b}	80.6 ± 1.1^{a}
	$1.72x10^{-3}$				
weight	7.058	3.54 ± 0.10^{ab}	3.24 ± 0.15^{b}	2.85 ± 0.35^{b}	4.24 ± 0.21^{a}
	$5.48x10^{-4}$				
condition	9.65	0.72 ± 0.02^{b}	0.72 ± 0.01^{b}	0.72 ± 0.01^{b}	0.81 ± 0.02^{a}
factor	**$4.9\ x10^{-5}$**				
NKA	6.54	10.58 ± 0.88^{b}	14.23 ± 1.40^{ab}	11.94 ± 0.94^{b}	17.61 ± 1.28^{a}
	$9.94\ x10^{-4}$				
α1a	0.61	1.07±0.16	1.27±0.21	1.31±0.17	1.35±0.06
	0.62				
α1b	1.5	1.04±0.13	0.85±0.11	0.84±0.16	1.16±0.10
	0.24				
GHR1	6.06	1.02 ± 0.10^{a}	1.05 ± 0.19^{a}	0.76 ± 0.12^{ab}	0.44 ± 0.06^{b}
	$4.16\ x10^{-3}$				
GR1	0.49	1.03±0.13	0.83±0.13	0.84±0.17	0.96±0.11
	0.69				
PrlR	0.18	1.03±0.16	1.21 ±0.11	1.20±0.25	1.11±0.15
	0.91				

In chapter 1, I showed that smolt physiology may be highly dynamic until marine entry – but changes were not explained spatially or temporally. It is also clear from my results in chapter 2, that the duration of the smolt window is finite and strongly influenced by temperature. Given changes in environment that are predicted, temperature regimes migrating smolts experience may have a profound influence on the successful

seawater entry and marine survival. If endocrine signals that stimulate smolting are lower in late migrating fish, it may suggest that these animals are near the end of the smolt window. Late migrants, therefore, may be at risk if environmental conditions are not favourable for maintaining smolt characteristics. It would appear to be imperative that late migrating fish reach seawater rapidly before physiological changes associated with the loss of smolt characteristics occur. Knowledge of temperatures that migrating smolts encounter, therefore, is important to understand potential effects on the smolt window.

Temperatures that Chilko Lake sockeye salmon smolts are likely to experience during migration were modelled using tagging and temperature data for early, peak and late migrants. Migration speed was calculated using data from an acoustic tagging study on Chilko Lake sockeye salmon smolts (Rechisky et al., 2013; Nathan Furey, University of British Columbia, Personal Communication). Average speed for migration downstream between acoustic receivers varied (Figure E.1; Table E.2). Based on travel time, temperatures experienced by fish at each location were estimated for locations between Chilko Lake and the mouth of the Fraser (Fig. E.1; Table E.3) and putative thermal regimes experienced by outmigrating Chilko smolts defined. Early migrants were considered to be fish leaving Chilko Lake on April 22, 2012 and were the group intercepted for the temperature treatment experiment (Chapter 2). Peak migrants were considered to be fish leaving Chilko Lake on April 27, 2012; the date when smolt emigration peaked in 2012. Thermal regimes for late migrants were modelled for fish leaving Chilko Lake on May 17, 2012, which coincided with removal of the FOC enumeration fence (Figure E.2).

Figure E.1: Map of the Fraser River watershed and coastal British Columbia, Canada. Open circles show location of receivers, and average speed between receivers is shown in Table E.2. Solid circles show the locations of temperature loggers, data source is shown in Table E.3.

Table E.2: Locations (Fig. E.1), distance between and average speed of migration between receivers.

Location of receiver	Distance (km)	Speed (km/day)
Mouth of Chilko (C) – Henry's Bridge (HB)	0 –15 = 15	50
Henry's Bridge (HB) – Siwash (S)	15 – 82 = 67	55
Siwash (S) – Farwell Canyon (FC)	82 –180 = 98	109
Farwell Canyon (FC) – Mission (M)	180 – 605 = 428	224
Mission (M) – Derby (D)	605 – 658 = 53	127
Derby (D) – Mouth of Fraser (MOF)	658 – 684 = 26	90

Table E.3: Location (Fig. E.1) of temperature loggers, the distance from the mouth of Chilko Lake and source of temperature data.

Location of temperature logger	Distance from mouth of Chilko Lake (km)	Data source
Enumeration Fence (F)	1	DFO Environmental Watch Program
Alexis Creek (AC)	106	DFO Environmental Watch Program
Big Creek (BC)	170	Water Survey of Canada
Texas Creek (TC)	362	Water Survey of Canada
Qualark (Q)	503	DFO Environmental Watch Program
Hope (H)	523	Water Survey of Canada
Steveston bouy (SB)	668	Environment Canada

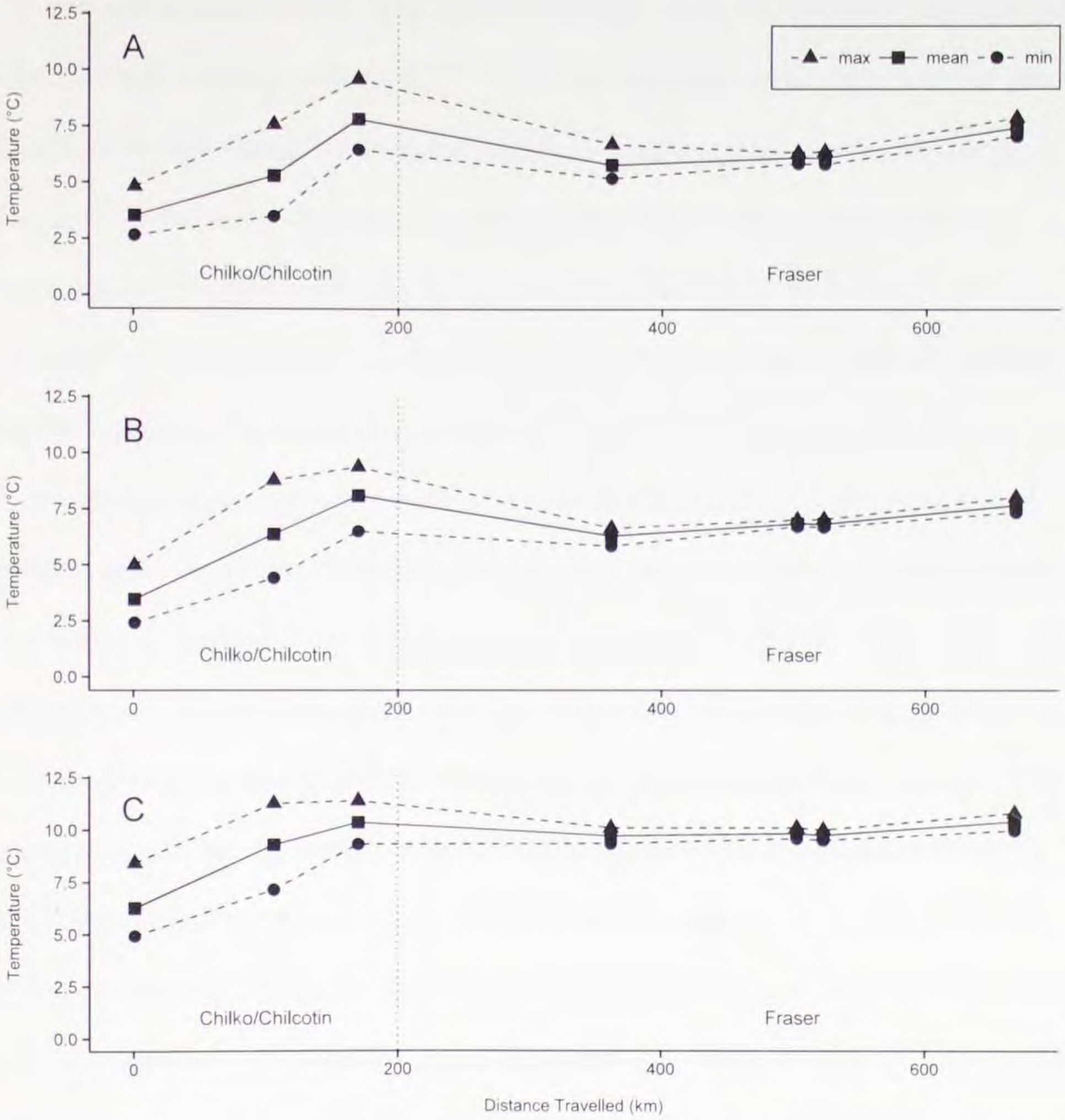

Figure E.2: Thermal Experience of Chilko smolts during freshwater migration in Chilko River, Chilcotin River and the Fraser River. Thermal experience of fish used for temperature experiment (A), peak outmigration of smolts (B), and the late migrators (C). Maximum (triangles), mean (squares) and minimum (circles) daily temperatures.

In chapter 2, smolts reverted back to parr state, only in the warmest treatment tank, where the mean temperature was 15.2 °C, and the maximum temperature recorded was 20.2 °C. The three thermal experiences modeled reveal that smolts are not migrating in temperatures that warm. The modeling data indicated that the highest temperatures migrating smolts might experience would be approximately 11.3 °C. From Chapter 2, fish subjected to temperatures as high as 16.1 °C in T2 did not show a decrease in NKA activity suggesting that saltwater tolerance did not decrease. Although, exceeding a threshold temperature appears to have driven the decline in NKA activity for sockeye salmon smolts from Chilko Lake, this does not rule out a potential effect of the thermal experience on duration of the smolt window. The accumulated thermal units (ATU) were also calculated during downstream migration between the fence and Steveston bouy (Fig. E.1). Early migrants had an ATU of 118 degree days, peak migrators had an ATU of 128 degree days, and the late migrators an ATU of 172 degree days. To put the values of ATU in context, the decline in NKA activity for the warmest group was not observed until approximately 200 degree days were exceeded. At 300 degree days, McCormick et al. (1997) observed a 50% decline in gill NKA activity for Atlantic salmon (*Salmo salar*) smolts. These results show that temperature either in the form of a threshold or ATU does not show a realistic risk in smolts. It would appear that projected temperature increases due to climate change are not likely to have much effect on the successful emigration of Chilko Lake smolts to the ocean.

Extrapolation of the data from Chapter 2, however, was based on smolts intercepted before the peak in numbers of migrating fish. It is possible that smolts leaving Chilko Lake later in the season could be a higher risk than fish migrating earlier in the

run. Interestingly, the warmest temperatures smolts were exposed to were early in migration in the Chilko and Chilcotin Rivers (Fig E.2). Consequently, migrants will accumulate more degree days during the first stages of migration when swimming speeds are also slower (Table E.2). The smaller volumes of the Chilko and Chilcotin Rivers compared to the Fraser River may also make these river systems more sensitive to warming trends associated with climate change. As well, the warmest temperatures were later in the spring; meaning late migrators experienced the higher temperatures. It is possible that later migrating smolts may already have an abbreviated smolt window if the endocrine stimulus for smolting is declining at the start of migration. Characterizing the effect of temperature on sockeye salmon smolts migrating from different times during the spring should be examined before I can conclude that there is no future impact of climate change on the success of Chilko Lake sockeye salmon smolts.

The thermal regimes that sockeye salmon currently experience as they emigrate from Chilko Lake should not impact successful transition to the marine environment. Recent tagging studies support my finding. Survival of Chilko Lake sockeye salmon smolts has been shown to be remarkably consistent among years. Mortality estimates are higher for fish during migration in freshwater than following seawater entry (Jeffries et al., 2014). Freshwater mortality appears to be largely confined to clear water tributaries upstream of the confluence between the Chilcotin River and the Fraser River; survival down the Fraser River mainstem is very high (Jefferies et al., 2014; Rechisky et al., 2013). The high disappearance rates in the Chilko and Chilcotin Rivers are likely attributed to predation; Furey et al. (2015) showed exclusive and intense feeding by bull trout (*Salvelinus confluentus)* on out-migrant smolts at Chilko River at the FOC enumeration

fence. Notable mortality did not occur between the mouth of the Fraser and the first seawater receiver (Northern Strait of Georgia) (Jefferies et al., 2014), suggesting successful transfer from fresh water to seawater.

Expanding these findings to the Fraser watershed, there may be implications on other populations. For instance, the Stuart and Stellako populations that have over 1000 km to migrate, and depart later in the season, may experience temperatures that induced parr-smolt reversion (David Patterson, personal communication). Similarly, other populations migrate in slower flow systems; with less elevation drop than in the Chilko system, fish in these systems will experience different water temperature regimes and potentially migration speeds. Therefore, it is not prudent to extrapolate the findings on Chilko smolts to other populations in the watershed without further investigation.

Adult spawning sockeye salmon experience higher mortality with warming temperature associated with climate change. Spawning migrations during warm water periods are associated with increased levels of mortality during migration and pre-spawn mortality on spawning grounds (Gilhousen, 1990; Macdonald et al., 2010; reviewed in Hinch and Martins 2011; Jefferies et al., 2012). Warming climates leading to warming water temperatures therefore lead to higher mortality during that phase of the salmon life cycle. The research presented here does not show the same kind of pressure on the freshwater to seawater phase of the lifecycle. The thermal experiences on Chilko juvenile migrants in 2012 are not at levels to induce the rapid reversion to parr. However, the mechanisms for causing high mortality are in place, and if the Fraser watershed continues to warm, this may be another phase of high temperature induced mortalities.

LIST OF REFERENCES

Adams BL, Zaugg WS and LR Mclain. 1973. Temperature effect on parr–smolt transformation in steelhead trout (*Salmo gairdneri*) as measured by gill sodium–potassium stimulated ATPase. *Comparative Biochemistry and Physiology* 44: 1333-1339.

Beacham TD, Lapointe M, Candy JR, McIntosh B, MacConnachie C, Tabata A, Kaukinen K , Deng L, Miller KM and RE Withler. 2004. Stock identification of Fraser River sockeye salmon using microsatellites and major histocompatibility complex variation. *Transactions of the American Fisheries Society* 133: 1117-1137.

Binsted GA and KI Ashley. 2006. Phosphorus loading to Kootenay Lake from the Kootenay and Duncan Rivers and experimental fertilization program. Prepared for the British Columbia Conservation Foundation.

Brauner CJ, Shrimpton JM and DJ Randall. 1992. Effect of short-duration seawater exposure on plasma ion concentrations and swimming performance in coho salmon (*Oncorhynchus kisutch*) parr. *Canadian Journal of Fisheries and Aquatic Sciences* 49: 2399-2405.

Burgner RL. 1998. Life history of sockeye salmon (*Oncorhynchus nerka)*. In Pacific Salmon Life Histories. Edited by C. Groot and L Margolis. UBC Press: Vancouver. Pp 3-101.

Cederholm JF, Kunze MD, Takeshi M and A Sibatani. 1999. Pacific salmon carcasses: essential contributions of nutrients and energy for aquatic and terrestrial ecosystems. *Fisheries* 24(10): 6-15.

Cohen BI. 2012. Cohen Commission of inquiry into the decline of sockeye salmon in the Fraser River - final report. The uncertain future of Fraser River sockeye, volume 1: the sockeye fishery. Minister of Public Works and Government Services Canada, Ottawa, BC.

Clarke WC, Shelbourn JE and JR Brett. 1978. Growth and adaptation to sea water in 'underyearling" sockeye (*Oncorhynchus nerka*) and coho (*O. kisutch*) salmon subjected to regimes of constant or changing temperature and day length. *Canadian Journal of Zoology* 56: 2413-2421.

Clark TD, NB Furey, EL Rechisky, MK Gale, KM Jeffries, AD Porter, MT Casselman, AG Lotto, DA Patterson, SJ Cooke, AP Farrell, DW Welch, and SG Hinch. Tracking the migration of wild sockeye salmon smolts to the ocean reveals distinct regions of nocturnal movement and high mortality. submitted

Clements S, Stahl TS and CB Schreck. 2012. Comparison of the behavior and survival of juvenile coho salmon and steelhead in a small coastal estuary. *Aquaculture* 362–363: 148-157.

Connor WP, Burge HL, Yearsley JR and TC Bjornn. 2003. Influence of flow and temperature on survival of wild subyearling fall Chinook salmon in the Snake River. *North American Journal of Fisheries Management* 23(2): 362-375.

Cooke SJ, Hinch SG, Farrell AP, Lapointe MF, Jones SRM, Macdonald JS, Patterson DA, Healey, MC and G Van Der Kraak. 2004. Abnormal migration timing and high en route mortality of sockeye salmon in the Fraser River, British Columbia. *Fisheries* 29(2): 22-33.

Cushman RM. 1985. Review of ecological effects of rapidly varying flows downstream from hydroelectric facilities. *North American Journal of Fisheries Management* 5(3A): 330-339.

Dittman A and T Quinn. 1996. Homing in Pacific salmon: mechanisms and ecological basis. *Journal of Experimental Biology* 199: 83-91.

Duston J, Saunders RL and DE Knox. 1991. Effects of increases in freshwater temperature on loss of smolt characteristics in Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences* 48: 164-169.

Evans DH, Piermarini PM and KP Choe. 2005. The multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. *Physiological Reviews* 85: 97-177.

Flores A-M, Shrimpton JM, Patterson DA, Hills JA, Cooke SJ, Yada T, Moriyama S, Hinch SG and AP Farrell. 2011. Physiological and molecular endocrine changes in maturing wild sockeye salmon, *Oncorhynchus nerka*, during ocean and river migration. *Journal of Comparative Physiology B* 182: 77-90.

Flores A-M and JM Shrimpton. 2012. Differential physiological and endocrine responses of rainbow trout, *Oncorhynchus mykiss*, transferred from fresh water to ion-poor or seawater. *General and Comparative Endocrinology* 175: 244-250.

Furey NB, Hinch SG, Lotto AG and DA Beauchamp. 2015. Extensive feeding on sockeye salmon *Oncorhynchus nerka* smolts by bull trout *Salvelinus confluentus* during initial outmigration into a small, unregulated and inland British Columbia river. *Journal of Fish Biology* 86: 392-401.

Gilhousen P. 1990. Prespawning mortalities of sockeye salmon in the Fraser River system and possible causal factors. *Int. Pac. Salmon Fish. Comm. Bull.* XXVI: 1-58.

Hague MJ, Ferrari MR, Miller JR, Patterson DA, Russell GL, Farrell AP and SG Hinch. 2011. Modelling the future hydroclimatology of the lower Fraser River and its impacts on the spawning migration survival of sockeye salmon. Global Change Biology 17: 87-98.

Handeland SO, Imsland AK, Ebbesson LOE, Nilsen TO, Hosfeld CD, Teien HChT and SO Stefansson. 2014. Osmoregulation and growth in offspring of wild Atlantic salmon at different temperatures. *Environmental Biology of Fishes* 97: 285-296.

Hinch SG, Cooke SJ, Healey MC and AP Farrell. 2005. Behavioral physiology of fish migrations: salmon as a model approach. In *Behavior and Physiology of Fish.* Fish Physiology Series. Vol. 24. Edited by KA Sloman, RW Wilson and S Balshine. Academic Press, London, pp 240-285.

Hinch SG and EG Martins. 2011. A review of potential climate change effects on survival of Fraser River sockeye salmon and an analysis of interannual trends in en route loss and pre-spawn mortality. Cohen Commission Technical Report, Vancouver, British Columbia. Available at: www.cohencommission.ca

Hiroi J and SD McCormick. 2012. New insights into gill ionocyte and ion transporter function in euryhaline and diadromous fish. *Respiratory Physiology & Neurobiology* 184: 257-268.

Hoar WS. 1988. The physiology of smolting salmonids. In: Hoar, WS, Randall, DJ (Eds), Fish Physiology, vol. XIB. Academic Press, San Diego, pp 275-343.

Jeffries KM, Hinch SG, Sierocinski T, Clark TD, Eliason EJ, Donaldson MR and S Li. 2012. Consequences of high temperatures and premature mortality on the transcriptome and blood physiology of wild adult sockeye salmon (*Oncorhynchus nerka*). *Ecology and Evolution* 2: 1747-1764.

Jeffries KM, Hinch SG, Gale MK, Clark TD, Lotto AG, Casselman MT, Li S, Rechisky EL, Porter AD, Welch DW and KM Miller. 2014. Immune response genes and pathogen presence predict migration survival in wild salmon smolts. *Molecular Ecology* 23: 5803-5815.

Jensen AJ, Finstad B, Fiske P, Hvidsten NA, Rikardsen AH and L Saksgård. 2012. Timing of smolt migration in sympatric populations of Atlantic salmon (*Salmo salar*), brown trout (*Salmo trutta*), and Arctic char (*Salvelinus alpinus*). *Canadian Journal of Fisheries and Aquatic Sciences* 69: 711-723.

Johnston NT, MacIsaac EA, Tschaplinski PJ and KJ Hall. 2004. Effects of the abundance of spawning sockeye salmon (*Oncorhynchus nerka*) on nutrients and algal biomass in forested streams. *Canadian Journal of Fisheries and Aquatic Sciences* 61: 384-403.

Jonsson B and N Jonsson. 2009. A review of the likely effects of climate change on anadromous Atlantic salmon *Salmo salar* and brown trout *Salmo trutta*, with particular reference to water temperature and flow. *Journal of Fish Biology* 75: 2381-2447.

Kiilerich P, Kristiansen K and SS Madsen. 2007. Hormone receptors in gill of smolting Atlantic salmon, Salmo salar: expression of growth hormone, prolactin, mineralocorticoid and glucocorticoid receptors and 11β-hydroxysteroid dehydrogenase type 2. *Journal of Endocrinology* 152: 295-303.

Lawson PW, Logerwell EA, Mantua NJ, Francis RC and VN Agostini. 2004. Environmental factors influencing freshwater survival and smolt production in Pacific Northwest coho salmon (*Oncorhynchus kisutch*). *Canadian Journal of Fisheries and Aquatic Sciences* 61: 360-373.

Livak KJ and TD Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. *Methods* 25: 402-408.

Macdonald JS, Patterson DA, Hague MJ and IC Guthrie. 2010. Modeling the influence of environmental factors on spawning migration mortality for sockeye salmon fisheries management in the Fraser River, British Columbia. *Transactions of the American Fisheries Society* 139: 768-782.

Madsen SS and ET Naamansen. 1989. Plasma ionic regulation and gill Na^+/K^+-ATPase changes during rapid transfer to sea water of yearling rainbow trout, *Salmo gairdneri*: time course and seasonal variation. *Journal of Fish Biology* 34: 829-84.

Madsen SS, Killerich P and CK Tipsmark. 2008. Multiplicity of expression on Na^+/K^+-ATPase α-subunit isoforms in the gill of Atlantic salmon (*Salmo salar*): cellular localization and absolute quantification in response to salinity change. *Journal of Experimental Biology* 212: 78-88.

McCartney MP, Sullivan C and M Acreman. 1999. Ecosystem impacts of large dams. Report for the IUCN-The World Conservation Union and the World Commission on Dams. 74 pp.

McCormick SD and RL Saunders. 1987. Preparatory physiological adaptations for marine life of salmonids: osmoregulation, growth, and metabolism. *American Fisheries Society Symposium* 1: 211-229.

McCormick SD. 1993. Methods for nonlethal gill biopsy and measurements of Na^+/K^+-ATPase activity. *Canadian Journal of Fisheries and Aquatic Sciences* 50: 656-658.

McCormick SD. 1995. Hormonal control of gill Na^+/K^+-ATPase and chloride cell function. In: *Fish Physiology, Vol. 14, Cellular and Molecular Approaches to Fish Ionic Regulation.* Ed.: C.M. Wood and T.J. Shuttleworth. Academic Press, New York. Pp 285-315.

McCormick SD, Shrimpton JM and JD Zydlewski. 1997. Temperature effects on osmoregulatory physiology of juvenile anadromous fish. In *Global Warming: Implications for Freshwater and Marine Fish.* Edited by CM Wood and DG McDonald. Cambridge University Press, Pp 260-271.

McCormick SD, Hansen LP, Quinn, TP and RL Saunders. 1998. Movement, migration and smolting in Atlantic salmon. Canadian Journal of Fisheries and Aquatic Sciences 55(Suppl. 1): 77-92.

McCormick SD, Cunjak RA, Dempson B, O'Dea MF and JB Carey. 1999. Temperature-related loss of smolt characteristics in Atlantic salmon (*Salmo salar*) in the wild. *Canadian Journal of Fisheries and Aquatic Sciences* 56:1649-1658.

McCormick SD, Moriyama S and BTh Björnsson. 2000. Low temperature limits the regulatory control of photoperiod: endocrinology of smolting in Atlantic salmon. American Journal of Physiology 278: R1352-R1361.

McCormick SD. 2001. Endocrine control of osmoregulation in teleost fish. *American Zoologist* 41: 781-794.

McCormick SD, Shrimpton JM, Moriyama, S. and BT Björnsson. 2002. Effects of an advanced temperature cycle on smolt development and endocrinology indicate that temperature is not a zeitgeber for smolting in Atlantic salmon. *Journal of Experimental Biology* 205: 3553-3560.

McCormick SD, Shrimpton JM, Moriyama, S. and BT Björnsson. 2007. Differential hormonal responses of Atlantic salmon parr and smolt to increased daylength: A possible developmental basis for smolting. Aquaculture 273: 337-344.

McCormick SD, Regish A, O'Dea, MF and JM Shrimpton. 2008. Are we missing a mineralocorticoid in teleost fish? Effects of cortisol, deoxycorticosterone and aldosterone on osmoregulation, gill Na^+/K^+-ATPase activity and isoform mRNA levels in Atlantic salmon. *General and Comparative Endocrinology* 157: 35-40.

McCormick SD, Regish AM and AK Christensen. 2009. Distinct freshwater and seawater isoforms of Na^+/K^+-ATPase in gill chloride cells of Atlantic salmon. *Journal of Experimental Biology* 212: 3994-4001.

McCormick SD. 2013. Smolt physiology and endocrinology. In: *Euryhaline Fishes (Fish Physiology Series)*. Vol. 32, eds. SD McCormick, AP Farrell and CJ Brauner, pp. 199-251. San Diego: Academic Press.

McCormick SD, Regish AM, Christensen AK and BTh Björnsson. 2013. Differential regulation of sodium-potassium pump isoforms during smolt development and seawater exposure of Atlantic salmon. *Journal of Experimental Biology* 216:1142-1151.

Morrison J, Quick MC and MGG Foreman. 2002. Climate change in the Fraser River watershed: flow and temperature projections. *The Journal of Hydrology* 263: 230-244.

Muir WD, Giorgi AE and TC Coley. 1994. Behavioral and physiological changes in yearling chinook salmon during hatchery residence and downstream migration. *Aquaculture* 127: 69-82.

Naiman RJ, Bilby RE, Schindler DE and JM Helfield. 2002. Pacific salmon, nutrients, and the dynamics of freshwater and riparian ecosystems. *Ecosystems* 5: 399-417.

Northcote TG and PA Larkin. 1956. Indices of productivity in British Columbia lakes. *Journal of the Fisheries Research Board of Canada* 13(4): 515-540.

Pacific Salmon Commission. 2009. [Press release]. Retrieved from: http://www.psc.org/NewsRel/2009/NewsRelease10.pdf.

Patterson DA, Macdonald JS, Skibo KM, Barnes DP, Guthrie I and J Hills. 2007. Reconstructing the summer thermal history for the lower Fraser River, 1941 to 2006, and implications for adult sockeye salmon (*Oncorhynchus nerka*) spawning migration. Canadian Technical Report of Fisheries and Aquatic Sciences 2724: 1-43.

Province of British Columbia. 2010. British Columbia Seafood Industry. Retrieved from: *bcseafood.ca/PDFs/YIR-2010.pdf*

Quinn TP, Volk EC and AP Hendry. 1999. Natural otolith microstructure patterns reveal precise homing to natal incubation sites by sockeye salmon (*Oncorhynchus nerka*). *Canadian Journal of Zoology* 77(5): 766-775.

Raymond HL. 1979. Effects of dams and impoundments on migrations of juvenile Chinook salmon and steelhead from the Snake River, 1966 to 1975. *Transactions of the American Fisheries Society* 108: 505-529.

Rechisky EL, Welch DW, Porter AD, Gale MK, Clark TD, Furey NB and SG Hinch. 2013. Telemetry-based estimates of early marine survival and residence time of juvenile Sockeye Salmon in the Strait of Georgia and discovery passage, 2012. In State of Physical, Biological, and Selected Fishery Resources of Pacific Canadian Marine Ecosystems in 2012 (Irvine, J. R. & Crawford, W. R., eds), pp. 131-134.

Reed TE, Schindler DE, Hague MJ, Patterson DA, Meir E, Waples RS and SG Hinch. 2011. Time to Evolve? Potential Evolutionary Responses of Fraser River Sockeye Salmon to Climate Change and Effects on Persistence. *PLoS ONE* 6(6): e20380.

Richards JG, Semple JW, Bystriansky JS and PM Schulte. 2003. Na^+/K^+-ATPase α-isoform switching in gills of rainbow trout (*Oncorhynchus mykiss*) during salinity transfer. *Journal of Experimental Biology* 206: 4475-4486.

Sathiyaa R and MM Vijayan. 2003. Autoregulation of glucocorticoid receptor by cortisol in rainbow trout hepatocytes. *American Journal of Physiology - Cell Physiology* 284: C1508-C1515.

Shrimpton JM, Bernier NJ and DJ Randall. 1994. Changes in cortisol dynamics in wild and hatchery-reared juvenile coho salmon (*Oncorhynchus kisutch*) during smoltification. *Canadian Journal of Fisheries and Aquatic Sciences* 51: 2179-2187.

Shrimpton JM, Devlin RH, McLean E, Byatt JC, Donaldson EM and DJ Randall. 1995. Increases in gill cytosolic corticosteroid receptor abundance and saltwater tolerance in juvenile coho salmon (*Oncorhynchus kisutch*) treated with growth hormone and placental lactogen. *General and Comparative Endocrinology* 98: 1-15.

Shrimpton JM. 1996. Relationship between size, gill corticosteroid receptors, Na^+K^+ATPase activity and smolting in juvenile coho salmon (*Oncorhynchus kisutch*) in autumn and spring. *Aquaculture* 147: 127-140.

Shrimpton JM and SD McCormick. 1998. Regulation of gill corticosteroid receptors in juvenile Atlantic salmon: Interaction effects of growth hormone with prolactin and triiodothyronine. *General and Comparative Endocrinology* 112: 262-274.

Shrimpton JM, Björnsson BTh and SD McCormick. 2000. Can Atlantic salmon smolt twice? Endocrine and biochemical changes during smolting. *Canadian Journal of Fisheries and Aquatic Sciences* 57: 1969-1976.

Shrimpton JM, Zydlewski JD and JW Heath. 2007. Effect of daily oscillation in temperature and increased suspended sediment on growth and smolting in juvenile chinook salmon, *Oncorhynchus tshawytscha*. *Aquaculture*. 273: 269-276.

Shrimpton JM. 2013. Seawater to freshwater transitions in diadromous fish. In: *Euryhaline Fishes (Fish Physiology Series)*. Vol. 32, eds. SD McCormick, AP Farrell and CJ Brauner, pp. 327-392. San Diego: Academic Press.

Shrimpton JM, Warren KD, Todd NL, McRae CJ, Glova GJ, Telmer KH and AD Clarke. 2014. Freshwater movement patterns by juvenile Pacific salmon *Oncorhynchus spp.* before they migrate to the ocean: Oh the places you'll go! *Journal of Fish Biology* 85: 987-1004.

Solomon DJ. 1978. Some observations on salmon smolt migration in a chalkstream. *Journal of Fish Biology*. 12: 571-574.

Stefansson SO, Björnsson BTh, Sundell K, Nyhammer G and SD McCormick. 2003. Physiological characteristics of wild Atlantic salmon post-smolts during estuarine and coastal migration. *Journal of Fish Biology* 63: 942-955.

Sykes GE, Johnson CJ and JM Shrimpton. 2009. Temperature and Flow Effects on Migration Timing of Chinook Salmon Smolts. *Transactions of the American Fisheries Society* 138(6): 1252-1265.

Sykes GE and JM Shrimpton. 2010. Effect of temperature and current manipulation on smolting in Chinook salmon (*Oncorhynchus tshawytscha*): the relationship between migratory behaviour and physiological development. *Canadian Journal of Fisheries and Aquatic Sciences* 67: 191-201.

Very NM, Kittilson JD, Norbeck LA and MA Sheridan. 2005. Isolation, characterization, and distribution of two cDNAs encoding for growth hormone receptor in rainbow trout (*Oncorhynchus mykiss*). *Comparative Biochemistry and Physiology B* 140: 615-628.

Wedemeyer GA, Saunders RL and WC Clarke. 1980. Environmental factors affecting smoltification and early marine survival of anadromous salmonids. *Marine Fisheries Review* 42: 1-14.

Zaugg WS and LR McLain. 1976. Influence of water temperature on gill sodium, potassium-stimulated ATPase activity in juvenile coho salmon (*Oncorhynchus kisutch*). *Comparative Biochemistry and Physiology* 54: 419-421.

Zaugg WS. 1981. Advanced photoperiod and water temperature effects on gill Na^+/K^+ adenosine triphosphatase activity and migration of juvenile steelhead (*Salmo gairdneri*). *Canadian Journal of Fisheries and Aquatic Sciences* 38: 758-764.

Zydlewski, G.B., Haro, A. and S.D. McCormick. 2005. Evidence for cumulative temperature as an initiating and terminating factor in downstream migratory behavior of Atlantic salmon (*Salmo salar*) smolts. *Canadian Journal of Fisheries and Aquatic Sciences* 62: 68-78.

Zydlewski GB and J Zydlewski. 2011. Gill Na^+/K^+-ATPase of Atlantic salmon smolts in freshwater is not a predictor of long- term growth in seawater. *Aquaculture* 362-363: 121-126.

APPENDICES

Appendix 2.1. Mean Gill Na^+/K^+-ATPase (NKA) activity (μmol ADP • mg^{-1} of protein • h^{-1}) relative to ATU. Significant differences are shown for values that do not have a common letter displayed (CLD) ($p < 0.05$).

Tank	ATU	Mean (NKA activity)	CLD
Fence1	0	10.58	ad
A2	15	12.28	abc
T1	20	13.95	abc
T2	28	13.25	abc
A2	30	12.20	abc
T1	42	13.39	abc
T3	44	11.71	ad
A2	47	11.83	abc
T1	65	13.06	abc
T2	68	12.65	abc
A2	84	12.55	abc
T1	90	13.07	abc
T3	102	12.07	abc
T2	105	12.85	abc
A2	107	14.24	abc
T1	115	14.03	abc
A2	126	12.08	abc
A1	129	14.16	abc
T2	144	14.47	ab
T1	150	12.22	abc
T3	159	9.36	cd
T1	177	14.85	a
T2	187	11.88	abc
T3	217	7.54	de
T2	237	12.77	abc
T2	272	10.80	bd
T3	280	5.62	ef
T3	347	4.73	f
T3	393	4.12	f

Appendix 2.2. Mean gill NKA α1a expression (relative to β –actin) relative to ATU. Significant differences are shown for values that do not have a common letter displayed (CLD) ($p < 0.05$).

Tank	ATU	Mean (α1a)	CLD
Fence	0	1.07	de
A2	15	3.06	ab
T2	28	1.93	bde
A2	30	3.16	ab
T3	44	3.39	a
A2	47	2.12	ade
A2	65	1.98	bde
T2	68	1.49	de
A2	84	2.26	ade
T3	102	1.33	de
T2	105	0.92	e
A2	107	1.42	de
A2	126	1.38	de
A1	129	1.89	bde
T2	144	1.54	cde
T3	159	1.31	de
T2	187	2.47	ad
T3	217	1.23	de
T2	237	2.01	ade
T2	272	3.02	abc
T3	280	2.28	ade
T3	347	1.66	bde
T3	393	2.57	ad

Appendix 2.3. Mean gill NKA α1b expression (relative to β –actin) relative to ATU. Significant differences are shown for values that do not have a common letter displayed (CLD) ($p < 0.05$).

Tank	ATU	Mean (α1b)	CLD
Fence	0	1.04	a
A2	15	1.03	a
T2	28	0.32	ce
A2	30	0.42	bce
T3	44	0.38	bce
A2	47	0.20	ce
A2	65	0.66	ac
T2	68	0.45	bce
A2	84	0.79	ab
T3	102	0.43	bce
T2	105	0.61	acd
A2	107	0.48	bce
A2	126	0.37	bce
A1	129	0.40	bce
T2	144	0.39	bce
T3	159	0.24	ce
T2	187	0.49	bce
T3	217	0.13	e
T2	237	0.16	de
T2	272	0.23	ce
T3	280	0.21	ce
T3	347	0.17	de
T3	393	0.17	ce

Appendix 2.4. Mean gill growth hormone 1 receptor (GHR1) mRNA expression relative to ATU. Significant differences are shown for values that do not have a common letter displayed (CLD) ($p < 0.05$).

Tank	ATU	Mean (GH)	CLD
Fence	0	1.02	a
A2	15	0.39	b
T2	28	0.55	b
A2	30	0.45	b
T3	44	0.31	b
A2	47	0.52	b
A2	65	0.57	b
T2	68	0.48	b
A2	84	0.51	b
T3	102	0.39	b
T2	105	0.36	b
A2	107	0.49	b
A2	126	0.44	b
A1	129	0.42	b
T2	144	0.41	b
T3	159	0.35	b
T2	187	0.30	b
T3	217	0.31	b
T2	237	0.54	b
T2	272	0.49	b
T3	280	0.31	b
T3	347	0.47	b
T3	393	0.45	b

Appendix 2.5: Mean gill prolactin receptor (PrlR) mRNA expression relative to ATU. Significant differences are shown for values that do not have a common letter displayed (CLD) ($p < 0.05$).

Tank	ATU	Mean (PrlR)	CLD
Fence	0	1.03	ac
A2	15	2.19	ab
T2	28	1.43	ac
A2	30	1.92	ac
T3	44	0.78	c
A2	47	1.31	ac
A2	65	1.64	ac
T2	68	1.27	ac
A2	84	1.68	ac
T3	102	1.45	ac
T2	105	0.98	ac
A2	107	1.36	ac
A2	126	1.35	ac
A1	129	2.09	ac
T2	144	0.95	ac
T3	159	0.94	ac
T2	187	0.91	bc
T3	217	1.07	ac
T2	237	2.27	a
T2	272	2.31	ab
T3	280	1.65	ac
T3	347	1.38	ac
T3	393	1.30	ac